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(54) Title: PRODUCTION OF GAMMA LINOLENIC ACID BY A Δ 6-DESATURASE (57) Abstract Linoleic acid is converted into γ -linolenic acid by the enzyme Δ 6-desaturase. The present invention is directed to isolated nucleic acids comprising the Δ 6-desaturase gene. More particularly, the isolated nucleic acid comprises the promoter, coding region and termination regions of the Δ 6-desaturase gene. The present invention provides recombinant constructions comprising the Δ 6-desaturase coding region in functional combination with heterologous regulatory sequences. The nucleic acids and recombinant constructions of the instant invention are useful in the production of GLA in transgenic organisms.		

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1 PRODUCTION OF GAMMA LINOLENIC ACID BY A $\Delta 6$ -DESATURASE

Linoleic acid (18:2) (LA) is transformed
into gamma linolenic acid (18:3) (GLA) by the enzyme
5 $\Delta 6$ -desaturase. When this enzyme, or the nucleic acid
encoding it, is transferred into LA-producing cells,
GLA is produced. The present invention provides
nucleic acids comprising the $\Delta 6$ -desaturase gene. More
specifically, the nucleic acids comprise the
10 promoters, coding regions and termination regions of
the $\Delta 6$ -desaturase genes. The present invention is
further directed to recombinant constructions
comprising a $\Delta 6$ -desaturase coding region in functional
combination with heterologous regulatory sequences.
15 The nucleic acids and recombinant constructions of the
instant invention are useful in the production of GLA
in transgenic organisms.

Unsaturated fatty acids such as linoleic
($C_{18}\Delta^{9,12}$) and α -linolenic ($C_{18}\Delta^{9,12,15}$) acids are essential
20 dietary constituents that cannot be synthesized by
vertebrates since vertebrate cells can introduce
double bonds at the Δ^9 position of fatty acids but
cannot introduce additional double bonds between the
 Δ^9 double bond and the methyl-terminus of the fatty
25 acid chain. Because they are precursors of other
products, linoleic and α -linolenic acids are essential
fatty acids, and are usually obtained from plant
sources. Linoleic acid can be converted by mammals
into γ -linolenic acid (GLA, $C_{18}\Delta^{6,9,12}$) which can in turn
30 be converted to arachidonic acid (20:4), a critically

1 important fatty acid since it is an essential
precursor of most prostaglandins.

The dietary provision of linoleic acid, by
virtue of its resulting conversion to GLA and
5 arachidonic acid, satisfies the dietary need for GLA
and arachidonic acid. However, a relationship has
been demonstrated between consumption of saturated
fats and health risks such as hypercholesterolemia,
atherosclerosis and other clinical disorders which
10 correlate with susceptibility to coronary disease,
while the consumption of unsaturated fats has been
associated with decreased blood cholesterol
concentration and reduced risk of atherosclerosis.
The therapeutic benefits of dietary GLA may result
15 from GLA being a precursor to arachidonic acid and
thus subsequently contributing to prostaglandin
synthesis. Accordingly, consumption of the more
unsaturated GLA, rather than linoleic acid, has
potential health benefits. However, GLA is not
20 present in virtually any commercially grown crop
plant.

Linoleic acid is converted into GLA by the
enzyme $\Delta 6$ -desaturase. $\Delta 6$ -desaturase, an enzyme of
more than 350 amino acids, has a membrane-bound domain
25 and an active site for desaturation of fatty acids.
When this enzyme is transferred into cells which
endogenously produce linoleic acid but not GLA, GLA is
produced. The present invention, by providing the
gene encoding $\Delta 6$ -desaturase, allows the production of
30 transgenic organisms which contain functional $\Delta 6$ -
desaturase and which produce GLA. In addition to

1 allowing production of large amounts of GLA, the
present invention provides new dietary sources of GLA.

The present invention is directed to
isolated $\Delta 6$ -desaturase genes. Specifically, the
5 isolated genes comprises the $\Delta 6$ -desaturase promoters,
coding regions, and termination regions.

The present invention is further directed to
expression vectors comprising the $\Delta 6$ -desaturase
promoter, coding region and termination region.

10 Yet another aspect of this invention is
directed to expression vectors comprising a $\Delta 6$ -
desaturase coding region in functional combination
with heterologous regulatory regions, i.e. elements
not derived from the $\Delta 6$ -desaturase gene.

15 Cells and organisms comprising the vectors
of the present invention, and progeny of such
organisms, are also provided by the present invention.

A further aspect of the present invention
provides isolated bacterial $\Delta 6$ -desaturase. An
20 isolated plant $\Delta 6$ -desaturase is also provided.

Yet another aspect of this invention
provides a method for producing plants with increased
gamma linolenic acid content.

A method for producing chilling tolerant
25 plants is also provided by the present invention.

Fig. 1 depicts the hydropathy profiles of
the deduced amino acid sequences of Synechocystis $\Delta 6$ -
desaturase (Panel A) and $\Delta 12$ -desaturase (Panel B).
Putative membrane spanning regions are indicated by
30 solid bars. Hydrophobic index was calculated for a

1 window size of 19 amino acid residues [Kyte, et al.
(1982) J. Molec. Biol. 157].

Fig. 2 provides gas liquid chromatography
profiles of wild type (Panel A) and transgenic (Panel
5 B) Anabaena.

Fig. 3 is a diagram of maps of cosmid cSy75,
cSy13 and Csy7 with overlapping regions and subclones.
The origins of subclones of Csy75, Csy75-3.5 and Csy7
are indicated by the dashed diagonal lines.
10 Restriction sites that have been inactivated are in
parentheses.

Fig. 4 provides gas liquid chromatography
profiles of wild type (Panel A) and transgenic (Panel
B) tobacco.

15 Fig. 5A depicts the DNA sequence of a Δ -6
desaturase cDNA isolated from borage.

Fig. 5B depicts the protein sequence of the
open reading frame in the isolated borage Δ -6
desaturase cDNA. Three amino acid motifs
20 characteristic of desaturases are indicated and are,
in order, lipid box, metal box 1, and metal box 2.

Fig. 6 is a dendrogram showing similarity of
the borage Δ 6-desaturase to other membrane-bound
desaturases. The amino acid sequence of the borage
25 Δ 6-desaturase was compared to other known desaturases
using Gene Works (IntelliGenetics). Numerical values
correlate to relative phylogenetic distances between
subgroups compared.

Fig. 7 is a restriction map of 221. Δ 6.NOS
30 and 121. Δ 6.NOS. In 221. Δ 6.NOS, the remaining portion

1 of the plasmid is pBI221 and in 121.Δ6.NOS, the
remaining portion of the plasmid is pBI121.

Fig. 8 provides gas liquid chromatography
profiles of mock transfected (Panel A) and 221.Δ6.NOS
5 transfected (Panel B) carrot cells. The positions of
18:2, 18:3 α, and 18:3 γ (GLA) are indicated.

Fig. 9 provides gas liquid chromatography
profiles of an untransformed tobacco leaf (Panel A)
and a tobacco leaf transformed with 121.Δ6.NOS. The
10 positions of 18:2, 18:3 α, 18:3 γ (GLA), and 18:4 are
indicated.

Fig. 10 provides gas liquid chromatography
profiles for untransformed tobacco seeds (Panel A) and
seeds of tobacco transformed with 121.Δ6.NOS. The
15 positions of 18:2, 18:3α and 18:3γ (GLA) are indicated.

The present invention provides isolated
nucleic acids encoding Δ6-desaturase. To identify a
nucleic acid encoding Δ6-desaturase, DNA is isolated
from an organism which produces GLA. Said organism
20 can be, for example, an animal cell, certain fungi
(e.g. Mortierella), certain bacteria (e.g.
Synechocystis) or certain plants (borage, Oenothera,
currants). The isolation of genomic DNA can be
accomplished by a variety of methods well-known to one
25 of ordinary skill in the art, as exemplified by
Sambrook et al. (1989) in Molecular Cloning: A
Laboratory Manual, Cold Spring Harbor, NY. The
isolated DNA is fragmented by physical methods or
enzymatic digestion and cloned into an appropriate
30 vector, e.g. a bacteriophage or cosmid vector, by any
of a variety of well-known methods which can be found

1 in references such as Sambrook et al. (1989).
Expression vectors containing the DNA of the present
invention are specifically contemplated herein. DNA
encoding $\Delta 6$ -desaturase can be identified by gain of
5 function analysis. The vector containing fragmented
DNA is transferred, for example by infection,
transconjugation, transfection, into a host organism
that produces linoleic acid but not GLA. As used
herein, "transformation" refers generally to the
10 incorporation of foreign DNA into a host cell.
Methods for introducing recombinant DNA into a host
organism are known to one of ordinary skill in the art
and can be found, for example, in Sambrook et al.
(1989). Production of GLA by these organisms (i.e.,
15 gain of function) is assayed, for example by gas
chromatography or other methods known to the
ordinarily skilled artisan. Organisms which are
induced to produce GLA, i.e. have gained function by
the introduction of the vector, are identified as
20 expressing DNA encoding $\Delta 6$ -desaturase, and said DNA is
recovered from the organisms. The recovered DNA can
again be fragmented, cloned with expression vectors,
and functionally assessed by the above procedures to
define with more particularity the DNA encoding $\Delta 6$ -
25 desaturase.

As an example of the present invention,
random DNA is isolated from the cyanobacteria
Synechocystis Pasteur Culture Collection (PCC) 6803,
American Type Culture Collection (ATCC) 27184, cloned
30 into a cosmid vector, and introduced by
transconjugation into the GLA-deficient cyanobacterium

1 Anabaena strain PCC 7120, ATCC 27893. Production of GLA from Anabaena linoleic acid is monitored by gas chromatography and the corresponding DNA fragment is isolated.

5 The isolated DNA is sequenced by methods well-known to one of ordinary skill in the art as found, for example, in Sambrook et al. (1989).

In accordance with the present invention, DNA molecules comprising $\Delta 6$ -desaturase genes have been
10 isolated. More particularly, a 3.588 kilobase (kb) DNA comprising a $\Delta 6$ -desaturase gene has been isolated from the cyanobacteria Synechocystis. The nucleotide sequence of the 3.588 kb DNA was determined and is shown in SEQ ID NO:1. Open reading frames defining
15 potential coding regions are present from nucleotide 317 to 1507 and from nucleotide 2002 to 3081. To define the nucleotides responsible for encoding $\Delta 6$ -desaturase, the 3.588 kb fragment that confers $\Delta 6$ -desaturase activity is cleaved into two subfragments,
20 each of which contains only one open reading frame. Fragment ORF1 contains nucleotides 1 through 1704, while fragment ORF2 contains nucleotides 1705 through 3588. Each fragment is subcloned in both forward and reverse orientations into a conjugal expression vector
25 (AM542, Wolk et al. [1984] Proc. Natl. Acad. Sci. USA 81, 1561) that contains a cyanobacterial carboxylase promoter. The resulting constructs (i.e. ORF1(F), ORF1(R), ORF2(F) and ORF2(R)) are conjugated to wild-type Anabaena PCC 7120 by standard methods (see, for
30 example, Wolk et al. (1984) Proc. Natl. Acad. Sci. USA 81, 1561). Conjugated cells of Anabaena are

1 identified as Neo^r green colonies on a brown
background of dying non-conjugated cells after two
weeks of growth on selective media (standard mineral
media BG11N + containing 30µg/ml of neomycin according
5 to Rippka et al., (1979) J. Gen Microbiol. 111, 1).
The green colonies are selected and grown in selective
liquid media (BG11N + with 15µg/ml neomycin). Lipids
are extracted by standard methods (e.g. Dahmer et al.,
(1989) Journal of American Oil Chemical Society 66,
10 543) from the resulting transconjugants containing the
forward and reverse oriented ORF1 and ORF2 constructs.
For comparison, lipids are also extracted from wild-
type cultures of Anabaena and Synechocystis. The
fatty acid methyl esters are analyzed by gas liquid
15 chromatography (GLC), for example with a Tracor-560
gas liquid chromatograph equipped with a hydrogen
flame ionization detector and a capillary column. The
results of GLC analysis are shown in Table 1.

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1 Table 1: Occurrence of C18 fatty acids in wild-type
and transgenic cyanobacteria

	SOURCE	18:0	18:1	18:2	γ 18:3	α 18:3	18:4
5	Anabaena (wild type)	+	+	+	-	+	-
	Anabaena + ORF1 (F)	+	+	+	-	+	-
	Anabaena + ORF1 (R)	+	+	+	-	+	-
	Anabaena + ORF2 (F)	+	+	+	+	+	+
10	Anabaena + ORF2 (R)	+	+	+	-	+	-
	Synechocystis (wild type)	+	+	+	+	-	-

As assessed by GLC analysis, GLA deficient
 15 Anabaena gain the function of GLA production when the
 construct containing ORF2 in forward orientation is
 introduced by transconjugation. Transconjugants
 containing constructs with ORF2 in reverse orientation
 to the carboxylase promoter, or ORF1 in either
 20 orientation, show no GLA production. This analysis
 demonstrates that the single open reading frame (ORF2)
 within the 1884 bp fragment encodes Δ 6-desaturase.
 The 1884 bp fragment is shown as SEQ ID NO:3. This is
 substantiated by the overall similarity of the
 25 hydropathy profiles between Δ 6-desaturase and Δ 12-
 desaturase [Wada et al. (1990) Nature 347] as shown in
 Fig. 1 as (A) and (B), respectively.

Also in accordance with the present
 invention, a cDNA comprising a Δ 6-desaturase gene from
 30 borage (Borago officinalis) has been isolated. The
 nucleotide sequence of the 1.685 kilobase (kb) cDNA

1 was determined and is shown in Fig. 5A (SEQ ID NO: 4).
The ATG start codon and stop codon are underlined.
The amino acid sequence corresponding to the open
reading frame in the borage delta 6-desaturase is
5 shown in Fig. 5B (SEQ ID NO: 5).

Isolated nucleic acids encoding $\Delta 6$ -
desaturase can be identified from other GLA-producing
organisms by the gain of function analysis described
above, or by nucleic acid hybridization techniques
10 using the isolated nucleic acid which encodes
Synechocystis or borage $\Delta 6$ -desaturase as a
hybridization probe. Both genomic and cDNA cloning
methods are known to the skilled artisan and are
contemplated by the present invention. The
15 hybridization probe can comprise the entire DNA
sequence disclosed as SEQ. ID NO:1 or SEQ. ID NO:4, or
a restriction fragment or other DNA fragment thereof,
including an oligonucleotide probe. Methods for
cloning homologous genes by cross-hybridization are
20 known to the ordinarily skilled artisan and can be
found, for example, in Sambrook (1989) and Beltz et
al. (1983) Methods in Enzymology 100, 266.

In another method of identifying a delta 6-
desaturase gene from an organism producing GLA, a cDNA
25 library is made from poly-A⁺ RNA isolated from
polysomal RNA. In order to eliminate hyper-abundant
expressed genes from the cDNA population, cDNAs or
fragments thereof corresponding to hyper-abundant
cDNAs genes are used as hybridization probes to the
30 cDNA library. Non hybridizing plaques are excised and
the resulting bacterial colonies are used to inoculate

1 liquid cultures and sequenced. For example, as a
means of eliminating other seed storage protein cDNAs
from a cDNA library made from borage polysomal RNA,
cDNAs corresponding to abundantly expressed seed
5 storage proteins are first hybridized to the cDNA
library. The "subtracted" DNA library is then used to
generate expressed sequence tags (ESTs) and such tags
are used to scan a data base such as GenBank to
identify potential desaturates.

10 Transgenic organisms which gain the function
of GLA production by introduction of DNA encoding Δ -
desaturase also gain the function of
octadecatetraenoic acid ($18:4^{\Delta 6,9,12,15}$) production.
Octadecatetraenoic acid is present normally in fish
15 oils and in some plant species of the Boraginaceae
family (Craig et al. [1964] J. Amer. Oil Chem. Soc.
41, 209-211; Gross et al. [1976] Can. J. Plant Sci.
56, 659-664). In the transgenic organisms of the
present invention, octadecatetraenoic acid results
20 from further desaturation of α -linolenic acid by $\Delta 6$ -
desaturase or desaturation of GLA by $\Delta 15$ -desaturase.

The 359 amino acids encoded by ORF2, i.e.
the open reading frame encoding Synechocystis $\Delta 6$ -
desaturase, are shown as SEQ. ID NO:2. The open
25 reading frame encoding the borage $\Delta 6$ -desaturase is
shown in SEQ ID NO: 5. The present invention further
contemplates other nucleotide sequences which encode
the amino acids of SEQ ID NO:2 and SEQ ID NO: 5. It
is within the ken of the ordinarily skilled artisan to
30 identify such sequences which result, for example,
from the degeneracy of the genetic code. Furthermore,

1 one of ordinary skill in the art can determine, by the
gain of function analysis described hereinabove,
smaller subfragments of the fragments containing the
open reading frames which encode $\Delta 6$ -desaturases.

5 The present invention contemplates any such
polypeptide fragment of $\Delta 6$ -desaturase and the nucleic
acids therefor which retain activity for converting LA
to GLA.

In another aspect of the present invention,
10 a vector containing a nucleic acid of the present
invention or a smaller fragment containing the
promoter, coding sequence and termination region of a
 $\Delta 6$ -desaturase gene is transferred into an organism,
for example, cyanobacteria, in which the $\Delta 6$ -desaturase
15 promoter and termination regions are functional.
Accordingly, organisms producing recombinant $\Delta 6$ -
desaturase are provided by this invention. Yet
another aspect of this invention provides isolated $\Delta 6$ -
desaturase, which can be purified from the recombinant
20 organisms by standard methods of protein purification.
(For example, see Ausubel et al. [1987] Current
Protocols in Molecular Biology, Green Publishing
Associates, New York).

Vectors containing DNA encoding $\Delta 6$ -
25 desaturase are also provided by the present invention.
It will be apparent to one of ordinary skill in the
art that appropriate vectors can be constructed to
direct the expression of the $\Delta 6$ -desaturase coding
sequence in a variety of organisms. Replicable
30 expression vectors are particularly preferred.
Replicable expression vectors as described herein are

1 DNA or RNA molecules engineered for controlled
expression of a desired gene, i.e. the $\Delta 6$ -desaturase
gene. Preferably the vectors are plasmids,
bacteriophages, cosmids or viruses. Shuttle vectors,
5 e.g. as described by Wolk et al. (1984) Proc. Natl.
Acad. Sci. USA, 1561-1565 and Bustos et al. (1991) J.
Bacteriol. 174, 7525-7533, are also contemplated in
accordance with the present invention. Sambrook et
al. (1989), Goeddel, ed. (1990) Methods in Enzymology
10 185 Academic Press, and Perbal (1988) A Practical
Guide to Molecular Cloning, John Wiley and Sons, Inc.,
provide detailed reviews of vectors into which a
nucleic acid encoding the present $\Delta 6$ -desaturase can be
inserted and expressed. Such vectors also contain
15 nucleic acid sequences which can effect expression of
nucleic acids encoding $\Delta 6$ -desaturase. Sequence
elements capable of effecting expression of a gene
product include promoters, enhancer elements, upstream
activating sequences, transcription termination
20 signals and polyadenylation sites. Both constitutive
and tissue specific promoters are contemplated. For
transformation of plant cells, the cauliflower mosaic
virus (CaMV) 35S promoter and promoters which are
regulated during plant seed maturation are of
25 particular interest. All such promoter and
transcriptional regulatory elements, singly or in
combination, are contemplated for use in the present
replicable expression vectors and are known to one of
ordinary skill in the art. The CaMV 35S promoter is
30 described, for example, by Restrepo et al. (1990)

1 Plant Cell 2, 987. Genetically engineered and mutated regulatory sequences are also contemplated.

The ordinarily skilled artisan can determine vectors and regulatory elements suitable for
5 expression in a particular host cell. For example, a vector comprising the promoter from the gene encoding the carboxylase of Anabaena operably linked to the coding region of $\Delta 6$ -desaturase and further operably linked to a termination signal from Synechocystis is
10 appropriate for expression of $\Delta 6$ -desaturase in cyanobacteria. "Operably linked" in this context means that the promoter and terminator sequences effectively function to regulate transcription. As a further example, a vector appropriate for expression
15 of $\Delta 6$ -desaturase in transgenic plants can comprise a seed-specific promoter sequence derived from helianthinin, napin, or glycinin operably linked to the $\Delta 6$ -desaturase coding region and further operably linked to a seed termination signal or the nopaline
20 synthase termination signal. As a still further example, a vector for use in expression of $\Delta 6$ -desaturase in plants can comprise a constitutive promoter or a tissue specific promoter operably linked to the $\Delta 6$ -desaturase coding region and further
25 operably linked to a constitutive or tissue specific terminator or the nopaline synthase termination signal.

In particular, the helianthinin regulatory elements disclosed in applicant's copending U.S.
30 Application Serial No. 682,354, filed April 8, 1991 and incorporated herein by reference, are contemplated

1 as promoter elements to direct the expression of the
Δ6-desaturase of the present invention.

Modifications of the nucleotide sequences or
regulatory elements disclosed herein which maintain
5 the functions contemplated herein are within the scope
of this invention. Such modifications include
insertions, substitutions and deletions, and
specifically substitutions which reflect the
degeneracy of the genetic code.

10 Standard techniques for the construction of
such hybrid vectors are well-known to those of
ordinary skill in the art and can be found in
references such as Sambrook et al. (1989), or any of
the myriad of laboratory manuals on recombinant DNA
15 technology that are widely available. A variety of
strategies are available for ligating fragments of
DNA, the choice of which depends on the nature of the
termini of the DNA fragments. It is further
contemplated in accordance with the present invention
20 to include in the hybrid vectors other nucleotide
sequence elements which facilitate cloning, expression
or processing, for example sequences encoding signal
peptides, a sequence encoding KDEL, which is required
for retention of proteins in the endoplasmic reticulum
25 or sequences encoding transit peptides which direct
Δ6-desaturase to the chloroplast. Such sequences are
known to one of ordinary skill in the art. An
optimized transit peptide is described, for example,
by Van den Broeck et al. (1985) Nature 313, 358.
30 Prokaryotic and eukaryotic signal sequences are

1 disclosed, for example, by Michaelis et al. (1982)
Ann. Rev. Microbiol. 36, 425.

A further aspect of the instant invention provides organisms other than cyanobacteria or plants
5 which contain the DNA encoding the $\Delta 6$ -desaturase of the present invention. The transgenic organisms contemplated in accordance with the present invention include bacteria, cyanobacteria, fungi, and plants and animals. The isolated DNA of the present invention
10 can be introduced into the host by methods known in the art, for example infection, transfection, transformation or transconjugation. Techniques for transferring the DNA of the present invention into such organisms are widely known and provided in
15 references such as Sambrook et al. (1989).

A variety of plant transformation methods are known. The $\Delta 6$ -desaturase gene can be introduced into plants by a leaf disk transformation-regeneration procedure as described by Horsch et al. (1985) Science
20 227, 1229. Other methods of transformation, such as protoplast culture (Horsch et al. (1984) Science 223, 496; DeBlock et al. (1984) EMBO J. 2, 2143; Barton et al. (1983) Cell 32, 1033) can also be used and are within the scope of this invention. In a preferred
25 embodiment plants are transformed with Agrobacterium-derived vectors. However, other methods are available to insert the $\Delta 6$ -desaturase genes of the present invention into plant cells. Such alternative methods include biolistic approaches (Klein et al. (1987)
30 Nature 327, 70), electroporation, chemically-induced DNA uptake, and use of viruses or pollen as vectors.

1 When necessary for the transformation
method, the $\Delta 6$ -desaturase genes of the present
invention can be inserted into a plant transformation
vector, e.g. the binary vector described by Bevan
5 (1984) Nucleic Acids Res. 12, 8111. Plant
transformation vectors can be derived by modifying the
natural gene transfer system of Agrobacterium
tumefaciens. The natural system comprises large Ti
(tumor-inducing)-plasmids containing a large segment,
10 known as T-DNA, which is transferred to transformed
plants. Another segment of the Ti plasmid, the vir
region, is responsible for T-DNA transfer. The T-DNA
region is bordered by terminal repeats. In the
modified binary vectors the tumor-inducing genes have
15 been deleted and the functions of the vir region are
utilized to transfer foreign DNA bordered by the T-DNA
border sequences. The T-region also contains a
selectable marker for antibiotic resistance, and a
multiple cloning site for inserting sequences for
20 transfer. Such engineered strains are known as
"disarmed" A. tumefaciens strains, and allow the
efficient transformation of sequences bordered by the
T-region into the nuclear genomes of plants.

Surface-sterilized leaf disks are inoculated
25 with the "disarmed" foreign DNA-containing A.
tumefaciens, cultured for two days, and then
transferred to antibiotic-containing medium.
Transformed shoots are selected after rooting in
medium containing the appropriate antibiotic,
30 transferred to soil and regenerated.

1 Another aspect of the present invention
provides transgenic plants or progeny of these plants
containing the isolated DNA of the invention. Both
monocotyledenous and dicotyledenous plants are
5 contemplated. Plant cells are transformed with the
isolated DNA encoding $\Delta 6$ -desaturase by any of the
plant transformation methods described above. The
transformed plant cell, usually in a callus culture or
leaf disk, is regenerated into a complete transgenic
10 plant by methods well-known to one of ordinary skill
in the art (e.g. Horsch et al. (1985) Science 227,
1129). In a preferred embodiment, the transgenic
plant is sunflower, oil seed rape, maize, tobacco,
peanut or soybean. Since progeny of transformed
15 plants inherit the DNA encoding $\Delta 6$ -desaturase, seeds
or cuttings from transformed plants are used to
maintain the transgenic plant line.

The present invention further provides a
method for providing transgenic plants with an
20 increased content of GLA. This method includes
introducing DNA encoding $\Delta 6$ -desaturase into plant
cells which lack or have low levels of GLA but contain
LA, and regenerating plants with increased GLA content
from the transgenic cells. In particular,
25 commercially grown crop plants are contemplated as the
transgenic organism, including, but not limited to,
sunflower, soybean, oil seed rape, maize, peanut and
tobacco.

The present invention further provides a
30 method for providing transgenic organisms which
contain GLA. This method comprises introducing DNA

1 encoding $\Delta 6$ -desaturase into an organism which lacks or
has low levels of GLA, but contains LA. In another
embodiment, the method comprises introducing one or
more expression vectors which comprise DNA encoding
5 $\Delta 12$ -desaturase and $\Delta 6$ -desaturase into organisms which
are deficient in both GLA and LA. Accordingly,
organisms deficient in both LA and GLA are induced to
produce LA by the expression of $\Delta 12$ -desaturase, and
GLA is then generated due to the expression of $\Delta 6$ -
10 desaturase. Expression vectors comprising DNA
encoding $\Delta 12$ -desaturase, or $\Delta 12$ -desaturase and $\Delta 6$ -
desaturase, can be constructed by methods of
recombinant technology known to one of ordinary skill
in the art (Sambrook *et al.*, 1989) and the published
15 sequence of $\Delta 12$ -desaturase (Wada *et al.* [1990] Nature
(London) 347, 200-203. In addition, it has been
discovered in accordance with the present invention
that nucleotides 2002-3081 of SEQ. ID NO:1 encode
cyanobacterial $\Delta 12$ -desaturase. Accordingly, this
20 sequence can be used to construct the subject
expression vectors. In particular, commercially grown
crop plants are contemplated as the transgenic
organism, including, but not limited to, sunflower,
soybean, oil seed rape, maize, peanut and tobacco.
25 The present invention is further directed to
a method of inducing chilling tolerance in plants.
Chilling sensitivity may be due to phase transition of
lipids in cell membranes. Phase transition
temperature depends upon the degree of unsaturation of
30 fatty acids in membrane lipids, and thus increasing
the degree of unsaturation, for example by introducing

1 Δ^6 -desaturase to convert LA to GLA, can induce or
improve chilling resistance. Accordingly, the present
method comprises introducing DNA encoding Δ^6 -
desaturase into a plant cell, and regenerating a plant
5 with improved chilling resistance from said
transformed plant cell. In a preferred embodiment,
the plant is a sunflower, soybean, oil seed rape,
maize, peanut or tobacco plant.

The following examples further illustrate
10 the present invention.

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EXAMPLE 1

Strains and Culture Conditions

Synechocystis (PCC 6803, ATCC 27184),
5 Anabaena (PCC 7120, ATCC 27893) and Synechococcus (PCC
7942, ATCC 33912) were grown photoautotrophically at
30°C in BG11N+ medium (Rippka et al. [1979] J. Gen.
Microbiol. 111, 1-61) under illumination of
incandescent lamps
10 ($60\mu\text{E.m}^{-2}.\text{S}^{-1}$). Cosmids and plasmids were selected and
propagated in Escherichia coli strain DH5 α on LB
medium supplemented with antibiotics at standard
concentrations as described by Maniatis et al. (1982)
Molecular Cloning: A Laboratory Manual, Cold Spring
15 Harbor Laboratory, Cold Spring, New York.

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EXAMPLE 2

Construction of Synechocystis Cosmid Genomic Library

5 Total genomic DNA from Synechocystis (PCC
6803) was partially digested with Sau3A and
fractionated on a sucrose gradient (Ausubel et al.
[1987] Current Protocols in Molecular Biology, Greene
Publishing Associates and Wiley Interscience, New
York). Fractions containing 30 to 40 kb DNA fragments
10 were selected and ligated into the dephosphorylated
BamHI site of the cosmid vector, pDUCA7 (Buikema et
al. [1991] J. Bacteriol. 173, 1879-1885). The ligated
DNA was packaged in vitro as described by Ausubel et
al. (1987), and packaged phage were propagated in E.
15 coli DH5 α containing the AvaI and Eco4711 methylase
helper plasmid, pRL528 as described by Buikema et al.
(1991). A total of 1152 colonies were isolated
randomly and maintained individually in twelve 96-well
microtiter plates.

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EXAMPLE 3

Gain-of-Function Expression of GLA in Anabaena

Anabaena (PCC 7120), a filamentous
5 cyanobacterium, is deficient in GLA but contains
significant amounts of linoleic acid, the precursor
for GLA (Figure 2; Table 2). The Synechocystis cosmid
library described in Example 2 was conjugated into
10 Anabaena (PCC 7120) to identify transconjugants that
produce GLA. Anabaena cells were grown to mid-log
phase in BG11N+ liquid medium and resuspended in the
same medium to a final concentration of approximately
2x10⁸ cells per ml. A mid-log phase culture of E.
15 coli RP4 (Burkardt et al. [1979] J. Gen. Microbiol.
114, 341-348) grown in LB containing ampicillin was
washed and resuspended in fresh LB medium. Anabaena
and RP4 were then mixed and spread evenly on BG11N+
plates containing 5% LB. The cosmid genomic library
20 was replica plated onto LB plates containing 50 µg/ml
kanamycin and 17.5 µg/ml chloramphenicol and was
subsequently patched onto BG11N+ plates containing
Anabaena and RP4. After 24 hours of incubation at
30°C, 30 µg/ml of neomycin was underlaid; and
incubation at 30°C was continued until transconjugants
25 appeared.

Individual transconjugants were isolated
after conjugation and grown in 2 ml BG11N+ liquid
medium with 15 µg/ml neomycin. Fatty acid methyl
esters were prepared from wild type cultures and
30 cultures containing pools of ten transconjugants as
follows. Wild type and transgenic cyanobacterial

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1 cultures were harvested by centrifugation and washed
twice with distilled water. Fatty acid methyl esters
were extracted from these cultures as described by
Dahmer et al. (1989) J. Amer. Oil. Chem. Soc. 66, 543-
5 548 and were analyzed by Gas Liquid Chromatography
(GLC) using a Tracor-560 equipped with a hydrogen
flame ionization detector and capillary column (30 m x
0.25 mm bonded FSOT Superox II, Alltech Associates
Inc., IL). Retention times and co-chromatography of
10 standards (obtained from Sigma Chemical Co.) were used
for identification of fatty acids. The average fatty
acid composition was determined as the ratio of peak
area of each C18 fatty acid normalized to an internal
standard.

15 Representative GLC profiles are shown in
Fig. 2. C18 fatty acid methyl esters are shown.
Peaks were identified by comparing the elution times
with known standards of fatty acid methyl esters and
were confirmed by gas chromatography-mass
20 spectrometry. Panel A depicts GLC analysis of fatty
acids of wild type Anabaena. The arrow indicates the
migration time of GLA. Panel B is a GLC profile of
fatty acids of transconjugants of Anabaena with
pAM542+1.8F. Two GLA producing pools (of 25 pools
25 representing 250 transconjugants) were identified that
produced GLA. Individual transconjugants of each GLA
positive pool were analyzed for GLA production; two
independent transconjugants, AS13 and AS75, one from
each pool, were identified which expressed significant
30 levels of GLA and which contained cosmids, cSy13 and
cSy75, respectively (Figure 3). The cosmids overlap

1 in a region approximately 7.5 kb in length. A 3.5 kb
NheI fragment of cSy75 was recloned in the vector
pDUCA7 and transferred to Anabaena resulting in gain-
of-function expression of GLA (Table 2).

5 Two NheI/Hind III subfragments (1.8 and 1.7
kb) of the 3.5 kb Nhe I fragment of cSy75-3.5 were
subcloned into "pBLUESCRIPT" (Stratagene) (Figure 3)
for sequencing. Standard molecular biology techniques
were performed as described by Maniatis et al. (1982)
10 and Ausubel et al. (1987). Dideoxy sequencing (Sanger
et al. [1977] Proc. Natl. Acad. Sci. USA 74, 5463-
5467) of pBS1.8 was performed with "SEQUENASE" (United
States Biochemical) on both strands by using specific
oligonucleotide primers synthesized by the Advanced
15 DNA Technologies Laboratory (Biology Department, Texas
A & M University). DNA sequence analysis was done
with the GCG (Madison, WI) software as described by
Devereux et al. (1984) Nucleic Acids Res. 12, 387-395.

Both NheI/HindIII subfragments were
20 transferred into a conjugal expression vector, AM542,
in both forward and reverse orientations with respect
to a cyanobacterial carboxylase promoter and were
introduced into Anabaena by conjugation.
Transconjugants containing the 1.8 kb fragment in the
25 forward orientation (AM542-1.8F) produced significant
quantities of GLA and octadecatetraenoic acid (Figure
2; Table 2). Transconjugants containing other
constructs, either reverse oriented 1.8 kb fragment or
forward and reverse oriented 1.7 kb fragment, did not
30 produce detectable levels of GLA (Table 2).

1 Figure 2 compares the C18 fatty acid profile
of an extract from wild type Anabaena (Figure 2A) with
that of transgenic Anabaena containing the 1.8 kb
fragment of cSy75-3.5 in the forward orientation
5 (Figure 2B). GLC analysis of fatty acid methyl esters
from AM542-1.8F revealed a peak with a retention time
identical to that of authentic GLA standard. Analysis
of this peak by gas chromatography-mass spectrometry
(GC-MS) confirmed that it had the same mass
10 fragmentation pattern as a GLA reference sample.
Transgenic Anabaena with altered levels of
polyunsaturated fatty acids were similar to wild type
in growth rate and morphology.

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1 Table 2 Composition of C18 Fatty Acids in Wild Type
and Transgenic Cyanobacteria

5	Strain	Fatty Acid (%)					
		18:0	18:1	18:2	18.3(α)	18.3(γ)	18.4
	Wild Type						
10	<i>Synechocystis</i> (sp. PCC6803)	13.6	4.5	54.5	-	27.3	-
	<i>Anabaena</i> (sp. PCC7120)	2.9	24.8	37.1	35.2	-	-
15	<i>Synechococcus</i> (sp. PCC7942)	20.6	79.4	-	-	-	-
	<i>Anabaena</i> Transconjugants						
	cSy75	3.8	24.4	22.3	9.1	27.9	12.5
	cSy75-3.5	4.3	27.6	18.1	3.2	40.4	6.4
20	pAM542 - 1.8F	4.2	13.9	12.1	19.1	25.4	25.4
	pAM542 - 1.8R	7.7	23.1	38.4	30.8	-	-
	pAM542 - 1.7F	2.8	27.8	36.1	33.3	-	-
	pAM542 - 1.7R	2.8	25.4	42.3	29.6	-	-
	<i>Synechococcus</i> Transformants						
25	pAM854	27.8	72.2	-	-	-	-
	pAM854 - Δ^{12}	4.0	43.2	46.0	-	-	-
	pAM854 - Δ^6	18.2	81.8	-	-	-	-
	pAM854 - $\Delta^6\Delta^{12}$	42.7	25.3	19.5	-	16.5	-
30	18:0, stearic acid; 18:1, oleic acid; 18:2, linoleic acid; 18:3(α), linolenic acid; 18:3(γ), γ -linolenic acid; 18:4, octadecatetraenoic acid						

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EXAMPLE 4**Transformation of Synechococcus
with $\Delta 6$ and $\Delta 12$ Desaturase Genes**

5 A third cosmid, cSy7, which contains a $\Delta 12$ -
desaturase gene, was isolated by screening the
Synechocystis genomic library with a oligonucleotide
synthesized from the published Synechocystis $\Delta 12$ -
desaturase gene sequence (Wada et al. [1990] Nature
10 (London) 347, 200-203). A 1.7 kb AvaI fragment from
this cosmid containing the $\Delta 12$ -desaturase gene was
identified and used as a probe to demonstrate that
cSy13 not only contains a $\Delta 6$ -desaturase gene but also
a $\Delta 12$ -desaturase gene (Figure 3). Genomic Southern
15 blot analysis further showed that both the $\Delta 6$ -and $\Delta 12$ -
desaturase genes are unique in the Synechocystis
genome so that both functional genes involved in C18
fatty acid desaturation are linked closely in the
Synechocystis genome.

20 The unicellular cyanobacterium Synechococcus
(PCC 7942) is deficient in both linoleic acid and
GLA(3). The $\Delta 12$ and $\Delta 6$ -desaturase genes were cloned
individually and together into pAM854 (Bustos et al.
[1991] J. Bacteriol. 174, 7525-7533), a shuttle vector
that contains sequences necessary for the integration
25 of foreign DNA into the genome of Synechococcus
(Golden et al. [1987] Methods in Enzymol. 153, 215-
231). Synechococcus was transformed with these gene
constructs and colonies were selected. Fatty acid
methyl esters were extracted from transgenic
30 Synechococcus and analyzed by GLC.

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1 Table 2 shows that the principal fatty acids
of wild type Synechococcus are stearic acid (18:0) and
oleic acid (18:1). Synechococcus transformed with
pAM854- Δ 12 expressed linoleic acid (18:2) in addition
5 to the principal fatty acids. Transformants with
pAM854- Δ 6 and Δ 12 produced both linoleate and GLA
(Table 1). These results indicated that Synechococcus
containing both Δ 12- and Δ 6-desaturase genes has
gained the capability of introducing a second double
10 bond at the Δ 12 position and a third double bond at
the Δ 6 position of C18 fatty acids. However, no
changes in fatty acid composition was observed in the
transformant containing pAM854- Δ 6, indicating that in
the absence of substrate synthesized by the Δ 12
15 desaturase, the Δ 6-desaturase is inactive. This
experiment further confirms that the 1.8 kb
NheI/HindIII fragment (Figure 3) contains both coding
and promoter regions of the Synechocystis Δ 6-
desaturase gene. Transgenic Synechococcus with
20 altered levels of polyunsaturated fatty acids were
similar to wild type in growth rate and morphology.

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EXAMPLE 5

Nucleotide Sequence of $\Delta 6$ -Desaturase

1 The nucleotide sequence of the 1.8 kb
5 fragment of cSy75-3.5 including the functional $\Delta 6$ -
desaturase gene was determined. An open reading frame
encoding a polypeptide of 359 amino acids was
identified (Figure 4). A Kyte-Doolittle hydropathy
analysis (Kyte et al. [1982] J. Mol. Biol. 157, 105-
10 132) identified two regions of hydrophobic amino acids
that could represent transmembrane domains (Figure
1A); furthermore, the hydropathic profile of the $\Delta 6$ -
desaturase is similar to that of the $\Delta 12$ -desaturase
gene (Figure 1B; Wada et al.) and $\Delta 9$ -desaturases
15 (Thiede et al. [1986] J. Biol. Chem. 261, 13230-
13235). However, the sequence similarity between the
Synechocystis $\Delta 6$ - and $\Delta 12$ -desaturases is less than 40%
at the nucleotide level and approximately 18% at the
amino acid level.

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EXAMPLE 6

Transfer of Cyanobacterial Δ^6 -Desaturase into Tobacco

The cyanobacterial Δ^6 -desaturase gene was mobilized into a plant expression vector and transferred to tobacco using Agrobacterium mediated gene transfer techniques. To ensure that the transferred desaturase is appropriately expressed in leaves and developing seeds and that the desaturase gene product is targeted to the endoplasmic reticulum or the chloroplast, various expression cassettes with Synechocystis Δ -desaturase open reading frame (ORF) were constructed. Components of these cassettes include: (i) a 35S promoter or seed specific promoter derived from the sunflower helianthinin gene to drive Δ^6 -desaturase gene expression in all plant tissues or only in developing seeds respectively, (ii) a putative signal peptide either from carrot extensin gene or sunflower helianthinin gene to target newly synthesized Δ^6 -desaturase into the ER, (iii) an ER lumen retention signal sequence (KDEL) at the COOH-terminal of the Δ^6 -desaturase ORF, and (iv) an optimized transit peptide to target Δ^6 desaturase into the chloroplast. The 35S promoter is a derivative of pRTL2 described by Restrepo et al. (1990). The optimized transit peptide sequence is described by Van de Broeck et al. (1985). The carrot extensin signal peptide is described by Chen et al. (1985) EMBO J. 9, 2145.

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Transgenic tobacco plants were produced containing a chimeric cyanobacterial desaturase gene,

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1 comprised of the Synechocystis Δ^6 desaturase gene fused
to an endoplasmic reticulum retention sequence (KDEL)
and extensin signal peptide driven by the CaMV 35S
promoter. PCR amplifications of transgenic tobacco
5 genomic DNA indicate that the Δ^6 desaturase gene was
incorporated into the tobacco genome. Fatty acid
methyl esters of leaves of these transgenic tobacco
plants were extracted and analyzed by Gas Liquid
Chromatography (GLC). These transgenic tobacco
10 accumulated significant amounts of GLA (Figure 4).
Figure 4 shows fatty acid methyl esters as determined
by GLC. Peaks were identified by comparing the
elution times with known standards of fatty acid
methyl ester. Accordingly, cyanobacterial genes
15 involved in fatty acid metabolism can be used to
generate transgenic plants with altered fatty acid
compositions.

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EXAMPLE 7

Construction of Borage cDNA library

Membrane bound polysomes were isolated from
5 borage seeds 12 days post pollination (12 DPP) using
the protocol established for peas by Larkins and
Davies (1975 Plant Phys. 55:749-756). RNA was
extracted from the polysomes as described by Mechler
(1987 Methods in Enzymology 152:241-248, Academic
10 Press).

Poly-A+ RNA was isolated from the membrane
bound polysomal RNA by use of Oligotex-dT beads
(Qiagen). Corresponding cDNA was made using
Stratagene's ZAP cDNA synthesis kit. The cDNA library
15 was constructed in the lambda ZAP II vector
(Stratagene) using the lambda ZAP II vector kit. The
primary library was packaged in Gigapack II Gold
packaging extract (Stratagene). The library was used
to generate expressed sequence tags (ESTs), and
20 sequences corresponding to the tags were used to scan
the GenBank database.

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EXAMPLE 8

Hybridization Protocol

Hybridization probes for screening the
5 storage cDNA library were generated by using random
primed DNA synthesis as described by Ausubel et al
(1994 Current Protocols in Molecular Biology, Wiley
Interscience, N.Y.) and corresponded to previously
identified abundantly expressed seed storage protein
10 cDNAs. Unincorporated nucleotides were removed by use
of a G-50 spin column (Boehringer Mannheim). Probe was
denatured for hybridization by boiling in a water bath
for 5 minutes, then quickly cooled on ice. Filters
for hybridization were prehybridized at 60°C for 2-4
15 hours in prehybridization solution (6XSSC [Maniatis et
al 1984 Molecular Cloning A Laboratory Manual, Cold
Spring Harbor Laboratory], 1X Denharts Solution, 0.05%
sodium pyrophosphate, 100 µg/ml denatured salmon sperm
DNA). Denatured probe was added to the hybridization
20 solution (6X SSC, 1X Denharts solution, 0.05% sodium
pyrophosphate, 100 µg/ml denatured salmon sperm DNA)
and incubated at 60°C with agitation overnight.
Filters were washed in 4x, 2x, and 1x SET washes for
15 minutes each at 60°C. A 20X SET stock solution is
25 3M NaCl, 0.4 M Tris base, 20 mM Na₂EDTA-2H₂O. The 4X
SET wash was 4X SET, 12.5 mM PO₄, pH 6.8 and 0.2% SDS.
The 2X SET wash was 2X SET, 12.5 mM PO₄, pH 6.8 and
0.2% SDS. The 1X SET wash was 1X SET, 12.5 mM PO₄, pH
6.8 and 0.2% SDS. Filters were allowed to air dry and
30 were then exposed to X-ray film for 24 hours with
intensifying screens at -80°C.

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EXAMPLE 9

Random sequencing of cDNAs from a borage seed
(12 DPP) membrane-bound polysomal library

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The borage cDNA library was plated at low density (500 pfu on 150 mm petri dishes). Highly prevalent seed storage protein cDNAs were "subtracted" by screening with the previously identified corresponding cDNAs. Non-hybridizing plaques were excised using Stratagene's excision protocol and reagents. Resulting bacterial colonies were used to inoculate liquid cultures and were either sequenced manually or by an ABI automated sequencer. Each cDNA was sequenced once and a sequence tag generated from 200-300 base pairs. All sequencing was performed by cycle sequencing (Epicentre). Over 300 ESTs were generated. Each sequence tag was compared to GenBank database by BLASTX computer program and a number of lipid metabolism genes, including the $\Delta 6$ -desaturase were identified.

Database searches with a cDNA clone designated mbp-65 using BLASTX with the GenBank database resulted in a significant match to the Synechocystis $\Delta 6$ -desaturase. It was determined however, that this clone was not a full length cDNA. A full length cDNA was isolated using mbp-65 to screen the borage membrane-bound polysomal library. The sequence of the isolated cDNA was determined (Fig. 5A, SEQ ID NO:4) and the protein sequence of the open reading frame (Fig. 5B, SEQ ID NO:5) was compared to other known desaturases using Geneworks

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1 (IntelligGenetics) protein alignment program (Fig. 2).
This alignment indicated that the cDNA was the borage
Δ6-desaturase gene.

Although similar to other known plant
5 desaturases, the borage delta 6-desaturase is distinct
as indicated in the dendrogram shown in Fig. 6.
Furthermore, comparison of the amino acid sequences
characteristic of desaturases, particularly those
proposed to be involved in metal binding (metal box 1
10 and metal box 2), illustrates the differences between
the borage delta 6-desaturase and other plant
desaturases (Table 3).

The borage delta 6-desaturase is
distinguished from the cyanobacterial form not only in
15 over all sequence (Fig. 6) but also in the lipid box,
metal box 1 and metal box 2 amino acid motifs (Table
3). As Table 3 indicates, all three motifs are novel
in sequence. Only the borage delta 6-desaturase metal
box 2 shown some relationship to the Synechocystis
20 delta-6 desaturase metal box 2.

In addition, the borage delta 6-desaturase
is also distinct from another borage desaturase gene,
the delta-12 desaturase. P1-81 is a full length cDNA
that was identified by EST analysis and shows high
25 similarity to the Arabidopsis delta-12 desaturase (Fad
2). A comparison of the lipid box, metal box 1 and
metal box 2 amino acid motifs (Table 3) in borage
delta 6 and delta-12 desaturases indicates that little
homology exists in these regions. The placement of
30 the two sequences in the dendrogram in Fig. 6
indicates how distantly related these two genes are.

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Table 3. Comparison of common amino acid motifs in membrane-bound desaturases

Desaturase	Lipid Box	Amino Acid Motif			Metal Box 1	Metal Box 2
Borage Δ^6	WIGHDAGH (SEQ. ID. NO: 6)	HNAHH (SEQ. ID. NO: 12)	FQIEHH (SEQ. ID. NO: 20)			
Synechocystis Δ^6	NVGHDANH (SEQ. ID. NO: 7)	HNYLHH (SEQ. ID. NO: 13)	HQVTHH (SEQ. ID. NO: 21)			
Arab. chloroplast Δ^{15}	VLGHDCGH (SEQ. ID. NO: 8)	HRTHH (SEQ. ID. NO: 14)	HVIHH (SEQ. ID. NO: 22)			
Rice Δ^{15}	VLGHDCGH (SEQ. ID. NO: 8)	HRTHH (SEQ. ID. NO: 14)	HVIHH (SEQ. ID. NO: 22)			
Glycine chloroplast Δ^{15}	VLGHDCGH (SEQ. ID. NO: 8)	HRTHH (SEQ. ID. NO: 14)	HVIHH (SEQ. ID. NO: 22)			
Arab. fad3 (Δ^{15})	VLGHDCGH (SEQ. ID. NO: 8)	HRTHH (SEQ. ID. NO: 14)	HVIHH (SEQ. ID. NO: 22)			
Brassica fad3 (Δ^{15})	VLGHDCGH (SEQ. ID. NO: 8)	HRTHH (SEQ. ID. NO: 14)	HVIHH (SEQ. ID. NO: 22)			
Borage Δ^{17} (P1-81)*	VIAHECGH (SEQ. ID. NO: 9)	HRRHH (SEQ. ID. NO: 15)	HVAHH (SEQ. ID. NO: 23)			
Arab. fad2 (Δ^{17})	VIAHECGH (SEQ. ID. NO: 9)	HRRHH (SEQ. ID. NO: 15)	HVAHH (SEQ. ID. NO: 23)			
Arab. chloroplast Δ^{17}	VIGHDCAH (SEQ. ID. NO: 10)	HDRHH (SEQ. ID. NO: 16)	HIPHH (SEQ. ID. NO: 24)			
Glycine plastid Δ^{17}	VIGHDCAH (SEQ. ID. NO: 10)	HDRHH (SEQ. ID. NO: 16)	HIPHH (SEQ. ID. NO: 24)			
Spinach plastidial n-6	VIGHDCAH (SEQ. ID. NO: 10)	HDQHH (SEQ. ID. NO: 17)	HIPHH (SEQ. ID. NO: 24)			
Synechocystis Δ^{17}	VVGHDCGH (SEQ. ID. NO: 11)	HDHHH (SEQ. ID. NO: 18)	HIPHH (SEQ. ID. NO: 24)			
Anabaena Δ^{12}	VLGHDCGH (SEQ. ID. NO: 8)	HNHHH (SEQ. ID. NO: 19)	HVPHH (SEQ. ID. NO: 25)			

*P1-81 is a full length cDNA which was identified by EST analysis and shows high similarity to the Arabidopsis $\Delta 12$ desaturase (fad2)

*P1-81 is a full length cDNA which was identified by EST analysis and shows high similarity to the Arabidopsis Δ^{12} desaturase (fad2)

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EXAMPLE 10

Construction of 222.1Δ⁶NOS for transient
and expression

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The vector pBI221 (Jefferson et al. 1987
EMBO J. 6:3901-3907) was prepared for ligation by
digestion with BamHI and EcoICR I (Promega) which
excises the GUS coding region leaving the 35S promoter
and NOS terminator intact. The borage Δ 6-desaturase
cDNA was excised from the Bluescript plasmid
10 (Stratagene) by digestion with BamHI and XhoI. The
XhoI end was made blunt by use of the Klenow fragment.
This fragment was then cloned into the BamHI/EcoICR I
sites of pBI221, yielding 221.Δ⁶NOS (Fig. 7). In
221.Δ⁶.NOS, the remaining portion (backbone) of the
15 restriction map depicted in Fig. 7 is pBI221.

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EXAMPLE 11

Construction of 121. Δ^6 .NOS for stable transformation

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5 The vector pBI121 (Jefferson et al. 1987
EMBO J. 6:3901-3907) was prepared for ligation by
digestion with BamHI and EcoICR I (Promega) which
excises the GUS coding region leaving the 35S promoter
and NOS terminator intact. The borage Δ^6 -desaturase
cDNA was excised from the Bluescript plasmid
10 (Stratagene) by digestion with BamHI and XhoI. The
XhoI end was made blunt by use of the Klenow fragment.
This fragment was then cloned into the BamHI/EcoICR I
sites of pBI121, yielding 121.1 Δ^6 NOS (Fig. 7). In
121. Δ^6 .NOS, the remaining portion (backbone) of the
15 restriction map depicted in Fig. 7 is pBI121.

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EXAMPLE 12
Transient Expression

5 All work involving protoplasts was performed
in a sterile hood. One ml of packed carrot suspension
cells were digested in 30 mls plasmolyzing solution
(25 g/l KCl, 3.5 g/l $\text{CaCl}_2 \cdot \text{H}_2\text{O}$, 10mM MES, pH 5.6 and
0.2 M mannitol) with 1% cellulase, 0.1% pectolyase,
and 0.1% dreisalase overnight, in the dark, at room
10 temperature. Released protoplasts were filtered
through a 150 μm mesh and pelleted by centrifugation
(100x g, 5 min.) then washed twice in plasmolyzing
solution. Protoplasts were counted using a double
chambered hemocytometer. DNA was transfected into the
15 protoplasts by PEG treatment as described by Nunberg
and Thomas (1993 Methods in Plant Molecular Biology
and Biotechnology, B.R. Glick and J.E. Thompson, eds.
pp. 241-248) using 10^6 protoplasts and 50-70 ug of
plasmid DNA (221.Δ6.NOS). Protoplasts were cultured
20 in 5 mls of MS media supplemented with 0.2M mannitol
and 3 μM 2,4-D for 48 hours in the dark with shaking.

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EXAMPLE 13

Stable transformation of tobacco

121.Δ⁶.NOS plasmid construction was used to transform tobacco (*Nicotiana tabacum* cv. xanthi) via Agrobacterium according to standard procedures (Horsh et al., 1985 Science 227: 1229-1231; Bogue et al., 1990 Mol. Gen. Genet. 221:49-57), except that initial transformants were selected on 100 ug/ml kanamycin.

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EXAMPLE 14

Preparation and analysis of
fatty acid methyl esters (FAMES)

1 Tissue from transfected protoplasts and
5 transformed tobacco plants was frozen in liquid
nitrogen and lyophilized overnight. FAMES were
prepared as described by Dahmer et al (1989 J. Amer.
Oil Chem. Soc. 66:543-548). In some cases, the
10 solvent was evaporated again, and the FAMES were
resuspended in ethyl acetate and extracted once with
deionized water to remove any water soluble
contaminants. The FAMES were analyzed by gas
chromatography (GC) on a J&W Scientific DB-wax column
(30 m length, 0.25 mm ID, 0.25 μ m film).

15 An example of a transient assay is shown in
Fig. 8 which represents three independent
transfections pooled together. The addition of the
borage $\Delta 6$ -desaturase cDNA corresponds with the
appearance of gamma linolenic acid (GLA) which is one
20 of the possible products of $\Delta 6$ -desaturase.

Figures 9 and 10 depict GC profiles of the
FAMES derived from leaf and seed tissue, respectively,
of control and transformed tobacco plants. Figure 9A
provides the profile of leaf tissue of wild-type
25 tobacco (xanthi); Figure 9B provides the profile of
leaf tissue from a tobacco plant transformed with the
borage Δ -6 desaturase under the transcriptional
control of the 35S CaMV promoter (pBI 121 Δ ⁶NOS).
Peaks correspond to 18:2, 18:3 γ (GLA), 18:3 α and 18:4
30 (octadecanonic acid). Figure 10A shows the GC profile
of seeds of a wild-type tobacco; Figure 10B shows the

1 profile of seed tissue of a tobacco plant transformed
with pBI 121 Δ ⁶NOS. Peaks correspond to 18:2,
18:3 γ (GLA) and 18:3 α .

5 The relative distribution of the C₁₈ fatty
acids in control and transgenic tobacco seeds is shown
in Table 4.

TABLE 4

	Fatty Acid	Xanthi	pBI121 Δ ⁶ NOS
10	18:0	4.0%	2.5%
	18:1	13%	13%
	18:2	82%	82%
	18:3 γ (GLA)	-	2.7%
15	18:3 α	0.82%	1.4%

20 The foregoing results demonstrate that GLA
is incorporated into the triacylglycerides of
transgenic tobacco leaves and seeds containing the
borage Δ 6-desaturase.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: Rhone-Poulenc Agrochimie
- (ii) TITLE OF INVENTION: PRODUCTION OF GAMMA LINOLENIC ACID BY A DELTA 6-DESATURASE
- (iii) NUMBER OF SEQUENCES: 25
- (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE: Scully, Scott, Murphy & Presser
 - (B) STREET: 400 Garden City Plaza
 - (C) CITY: Garden City
 - (D) STATE: New York
 - (E) COUNTRY: United States
 - (F) ZIP: 11530
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #1.25
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER:
 - (B) FILING DATE: 30-DEC-1994
 - (C) CLASSIFICATION:
- (viii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: Presser, Leopold
 - (B) REGISTRATION NUMBER: 19,827
 - (C) REFERENCE/DOCKET NUMBER: 8383ZYXW
- (ix) TELECOMMUNICATION INFORMATION:
 - (A) TELEPHONE: (516) 742-4343
 - (B) TELEFAX: (516) 742-4366
 - (C) TELEX: 230 901 SANS UR

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 3588 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: both
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)

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(ix) FEATURE:

(A) NAME/KEY: CDS
(B) LOCATION: 2002..3081

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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CACCTTGCCA GACCACGTTA GTTTGAGTGT TTCCGCCCTG GCGGCCCCGA TTTTTCCTT	180
TGCGGCTTTG GGCAATCAGG CGATCGGGCA ATTGCGTTTG TTTGACCAGA CTTGGCCCAT	240
TCAGGAAATT GTCATTACCC AAGACCATCC CTGGCTCAAT TTACCCCTGG CGGATTTATG	300
GGATGATCCG AGCCGAATGT TGATCTATTA CCTACCGGCC CACAGTGAAA CGGATTTAGT	360
AGGCGCAGTG GTGAATAATT TAACGTTGCA ATCTGGGGAC CATTTAATAG TGGGACAAAA	420
ACCCCAACCC AAGACCAAAC GGCGATCGCC TTGGCGCAA TTTTCCAAAC TGATTACCAA	480
CCTGCGGGAG TATCAGCGGT ATGTCCAACA GGTGATATGG GTGGTGTGTG TTTTATTGTT	540
GATGATTTTT CTGGCCACCT TCATCTACGT TTCCATTGAT CAACATATTG CCCCAGTGGA	600
CGCGTTGTAT TTTTCCGTGG GCATGATTAC CGGGGCCGGT GGCAAGGAAG AGGTGGCCGA	660
AAAGTCCCCC GATATCATCA AAGTATTCAC AGTGGTGATG ATGATCGCCG GGGCGGGGGT	720
GATTGGTATT TGTTATGCCC TACTGAATGA TTTCATCCTT GGCAGTCGCT TTAGTCAGTT	780
TTTGGATGCG GCCAAGTTAC CCGATCGCCA TCACATCATC ATTTGTGGGC TGGGGGGAGT	840
GAGCATGGCC ATTATTGAAG AGTTAATTCA CCAGGGCCAT GAAATTGTGG TAATCGAAAA	900
GGATACAGAT AATCGTTTCT TGCATACGGC CCGCTCCCTG GGGGTGCCCC TAATTGTGGA	960
GGATGCCCCG CTAGAAAGAA CGTTGGCCTG CGCCAATATC AACCGAGCCG AAGCCATTGT	1020
GGTGGCCACC AGCGACGACA CCGTTAACTT GGAAATTGGC CTAAGTGGCA AGGCGATCGC	1080
CCCTAGCCTG CCAGTGGTGT TGCGTTGCCA GGATGCCAG TTTAGCCTGT CCCTGCAGGA	1140
AGTATTTGAA TTTGAAACGG TGCTTTGTCC GGCAGGAATTG GCCACCTATT CCTTTGCGGC	1200
GGCGGCCCTG GGGGGCAAAA TTTTGGGCAA CGGCATGACC GATGATTTGC TGTGGGTAGC	1260
CCTAGCCACC TTAATCACTC CTAACCATCC CTTTGCCGAC CAATTGGTTA AAATTGCAGC	1320
CCAAAAGTCT GATTTCTGTT CCCTCTATCT AGAACGGGGT GGCAAAACCA TCCATAGCTG	1380
GGAATTATTG GGTACCCATC TCGACTCTGG AGACGTGTTG TATTTAACCA TGCCCGCCAC	1440
TGCCCTAGAG CAACTTTGGC GATCGCCCCG TGCCACTGCT GATCCTCTGG ACTCTTTTTT	1500

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GGTTTAGCAT	GGGGGGATGG	AACTCTTGAC	TCGGCCCAAT	GGTGATCAAG	AAAGAACGCT	1560
TTGTCTATGT	TTAGTATTTT	TAAGTTAACC	AACAGCAGAG	GATAACTTCC	AAAAGAAATT	1620
AAGCTCAAAA	AGTAGCAAAA	TAAGTTTAAT	TCATAACTGA	GTTTTACTGC	TAAACAGCGG	1680
TGCAAAAAAG	TCAGATAAAA	TAAAGCTTC	ACTTCGGTTT	TATATTGTGA	CCATGGTTCC	1740
CAGGCATCTG	CTCTAGGGAG	TTTTTCCGCT	GCCTTTAGAG	AGTATTTTCT	CCAAGTCGGC	1800
TAACTCCCCC	ATTTTTAGGC	AAAATCATAT	ACAGACTATC	CCAATATTGC	CAGAGCTTTG	1860
ATGACTCACT	GTAGAAGGCA	GAATAAAATT	CTAGCAATGG	ACTCCCAGTT	GGAATAAATT	1920
TTTAGTCTCC	CCCGGCGCTG	GAGTTTTTTT	GTAGTTAATG	GCGGTATAAT	GTGAAAGTTT	1980
TTTATCTATT	TAAATTTATA	A	ATG CTA ACA GCG GAA AGA ATT AAA TTT ACC			2031
			Met Leu Thr Ala Glu Arg Ile Lys Phe Thr			
			1 5 10			
CAG AAA CGG GGG TTT CGT CGG GTA CTA AAC CAA CGG GTG GAT GCC TAC						2079
Gln Lys Arg Gly Phe Arg Arg Val Leu Asn Gln Arg Val Asp Ala Tyr						
TTT GCC GAG CAT GGC CTG ACC CAA AGG GAT AAT CCC TCC ATG TAT CTG						2127
Phe Ala Glu His Gly Leu Thr Gln Arg Asp Asn Pro Ser Met Tyr Leu						
AAA ACC CTG ATT ATT GTG CTC TGG TTG TTT TCC GCT TGG GCC TTT GTG						2175
Lys Thr Leu Ile Ile Val Leu Trp Leu Phe Ser Ala Trp Ala Phe Val						
CTT TTT GCT CCA GTT ATT TTT CCG GTG CGC CTA CTG GGT TGT ATG GTT						2223
Leu Phe Ala Pro Val Ile Phe Pro Val Arg Leu Leu Gly Cys Met Val						
TTG GCG ATC GCC TTG GCG GCC TTT TCC TTC AAT GTC GGC CAC GAT GCC						2271
Leu Ala Ile Ala Leu Ala Ala Phe Ser Phe Asn Val Gly His Asp Ala						
AAC CAC AAT GCC TAT TCC TCC AAT CCC CAC ATC AAC CGG GTT CTG GGC						2319
Asn His Asn Ala Tyr Ser Ser Asn Pro His Ile Asn Arg Val Leu Gly						
ATG ACC TAC GAT TTT GTC GGG TTA TCT AGT TTT CTT TGG CGC TAT CGC						2367
Met Thr Tyr Asp Phe Val Gly Leu Ser Ser Phe Leu Trp Arg Tyr Arg						
CAC AAC TAT TTG CAC CAC ACC TAC ACC AAT ATT CTT GGC CAT GAC GTG						2415
His Asn Tyr Leu His His Thr Tyr Thr Asn Ile Leu Gly His Asp Val						
GAA ATC CAT GGA GAT GGC GCA GTA CGT ATG AGT CCT GAA CAA GAA CAT						2463
Glu Ile His Gly Asp Gly Ala Val Arg Met Ser Pro Glu Gln Glu His						

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Val	Gly	Ile	Tyr	Arg	Phe	Gln	Gln	Phe	Tyr	Ile	Trp	Gly	Leu	Tyr	Leu	
155					160					165					170	
TTC	ATT	CCC	TTT	TAT	TGG	TTT	CTC	TAC	GAT	GTC	TAC	CTA	GTG	CTT	AAT	2559
Phe	Ile	Pro	Phe	Tyr	Trp	Phe	Leu	Tyr	Asp	Val	Tyr	Leu	Val	Leu	Asn	
				175					180					185		
AAA	GGC	AAA	TAT	CAC	GAC	CAT	AAA	ATT	CCT	CCT	TTC	CAG	CCC	CTA	GAA	2607
Lys	Gly	Lys	Tyr	His	Asp	His	Lys	Ile	Pro	Pro	Phe	Gln	Pro	Leu	Glu	
			190					195					200			
TTA	GCT	AGT	TTG	CTA	GGG	ATT	AAG	CTA	TTA	TGG	CTC	GGC	TAC	GTT	TTC	2655
Leu	Ala	Ser	Leu	Leu	Gly	Ile	Lys	Leu	Leu	Trp	Leu	Gly	Tyr	Val	Phe	
		205					210					215				
GGC	TTA	CCT	CTG	GCT	CTG	GGC	TTT	TCC	ATT	CCT	GAA	GTA	TTA	ATT	GGT	2703
Gly	Leu	Pro	Leu	Ala	Leu	Gly	Phe	Ser	Ile	Pro	Glu	Val	Leu	Ile	Gly	
	220					225					230					
GCT	TCG	GTA	ACC	TAT	ATG	ACC	TAT	GGC	ATC	GTG	GTT	TGC	ACC	ATC	TTT	2751
Ala	Ser	Val	Thr	Tyr	Met	Thr	Tyr	Gly	Ile	Val	Val	Cys	Thr	Ile	Phe	
235					240					245					250	
ATG	CTG	GCC	CAT	GTG	TTG	GAA	TCA	ACT	GAA	TTT	CTC	ACC	CCC	GAT	GGT	2799
Met	Leu	Ala	His	Val	Leu	Glu	Ser	Thr	Glu	Phe	Leu	Thr	Pro	Asp	Gly	
				255					260					265		
GAA	TCC	GGT	GCC	ATT	GAT	GAC	GAG	TGG	GCT	ATT	TGC	CAA	ATT	CGT	ACC	2847
Glu	Ser	Gly	Ala	Ile	Asp	Asp	Glu	Trp	Ala	Ile	Cys	Gln	Ile	Arg	Thr	
			270					275					280			
ACG	GCC	AAT	TTT	GCC	ACC	AAT	AAT	CCC	TTT	TGG	AAC	TGG	TTT	TGT	GGC	2895
Thr	Ala	Asn	Phe	Ala	Thr	Asn	Asn	Pro	Phe	Trp	Asn	Trp	Phe	Cys	Gly	
		285					290					295				
GGT	TTA	AAT	CAC	CAA	GTT	ACC	CAC	CAT	CTT	TTC	CCC	AAT	ATT	TGT	CAT	2943
Gly	Leu	Asn	His	Gln	Val	Thr	His	His	Leu	Phe	Pro	Asn	Ile	Cys	His	
	300					305					310					
ATT	CAC	TAT	CCC	CAA	TTG	GAA	AAT	ATT	ATT	AAG	GAT	GTT	TGC	CAA	GAG	2991
Ile	His	Tyr	Pro	Gln	Leu	Glu	Asn	Ile	Ile	Lys	Asp	Val	Cys	Gln	Glu	
315					320					325					330	
TTT	GGT	GTG	GAA	TAT	AAA	GTT	TAT	CCC	ACC	TTC	AAA	GCG	GCG	ATC	GCC	3039
Phe	Gly	Val	Glu	Tyr	Lys	Val	Tyr	Pro	Thr	Phe	Lys	Ala	Ala	Ile	Ala	
				335					340					345		
TCT	AAC	TAT	CGC	TGG	CTA	GAG	GCC	ATG	GGC	AAA	GCA	TCG	TGACATTGCC			3088
Ser	Asn	Tyr	Arg	Trp	Leu	Glu	Ala	Met	Gly	Lys	Ala	Ser				
			350					355				360				
TTGGGATTGA	AGCAAAATGG	CAAAATCCCT	CGTAAATCTA	TGATCGAAGC	CTTTCTGTTG											3148
CCCGCCGACC	AAATCCCCGA	TGCTGACCAA	AGGTTGATGT	TGGCATTGCT	CCAAACCCAC											3208

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TTTGAGGGGG TTCATTGGCC GCAGTTTCAA GCTGACCTAG GAGGCAAAGA TTGGGTGATT 3268
TTGCTCAAAT CCGCTGGGAT ATTGAAAGGC TTCACCACCT TTGGTTTCTA CCCTGCTCAA 3328
TGGGAAGGAC AAACCGTCAG AATTGTTTAT TCTGGTGACA CCATCACCGA CCCATCCATG 3388
TGGTCTAACC CAGCCCTGGC CAAGGCTTGG ACCAAGGCCA TGCAAATTCT CCACGAGGCT 3448
AGGCCAGAAA AATTATATTG GCTCCTGATT TCTTCCGGCT ATCGCACCTA CCGATTTTTG 3508
AGCATTTTTG CCAAGGAATT CTATCCCCAC TATCTCCATC CCACTCCCCC GCCTGTACAA 3568
AATTTTATCC ATCAGCTAGC 3588

```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 359 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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Met Leu Thr Ala Glu Arg Ile Lys Phe Thr Gln Lys Arg Gly Phe Arg
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Arg Val Leu Asn Gln Arg Val Asp Ala Tyr Phe Ala Glu His Gly Leu
          20           25           30
Thr Gln Arg Asp Asn Pro Ser Met Tyr Leu Lys Thr Leu Ile Ile Val
          35           40           45
Leu Trp Leu Phe Ser Ala Trp Ala Phe Val Leu Phe Ala Pro Val Ile
          50           55           60
Phe Pro Val Arg Leu Leu Gly Cys Met Val Leu Ala Ile Ala Leu Ala
          65           70           75           80
Ala Phe Ser Phe Asn Val Gly His Asp Ala Asn His Asn Ala Tyr Ser
          85           90           95
Ser Asn Pro His Ile Asn Arg Val Leu Gly Met Thr Tyr Asp Phe Val
          100          105          110
Gly Leu Ser Ser Phe Leu Trp Arg Tyr Arg His Asn Tyr Leu His His
          115          120          125
Thr Tyr Thr Asn Ile Leu Gly His Asp Val Glu Ile His Gly Asp Gly
          130          135          140
Ala Val Arg Met Ser Pro Glu Gln Glu His Val Gly Ile Tyr Arg Phe
          145          150          155          160

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Gln Gln Ph Tyr Ile Trp Gly Leu Tyr Leu Phe Ile Pro Phe Tyr Trp
 165 170 175
 Phe Leu Tyr Asp Val Tyr Leu Val Leu Asn Lys Gly Lys Tyr His Asp
 180 185 190
 His Lys Ile Pro Pro Phe Gln Pro Leu Glu Leu Ala Ser Leu Leu Gly
 195 200 205
 Ile Lys Leu Leu Trp Leu Gly Tyr Val Phe Gly Leu Pro Leu Ala Leu
 210 215 220
 Gly Phe Ser Ile Pro Glu Val Leu Ile Gly Ala Ser Val Thr Tyr Met
 225 230 235 240
 Thr Tyr Gly Ile Val Val Cys Thr Ile Phe Met Leu Ala His Val Leu
 245 250 255
 Glu Ser Thr Glu Phe Leu Thr Pro Asp Gly Glu Ser Gly Ala Ile Asp
 260 265 270
 Asp Glu Trp Ala Ile Cys Gln Ile Arg Thr Thr Ala Asn Phe Ala Thr
 275 280 285
 Asn Asn Pro Phe Trp Asn Trp Phe Cys Gly Gly Leu Asn His Gln Val
 290 295 300
 Thr His His Leu Phe Pro Asn Ile Cys His Ile His Tyr Pro Gln Leu
 305 310 315 320
 Glu Asn Ile Ile Lys Asp Val Cys Gln Glu Phe Gly Val Glu Tyr Lys
 325 330 335
 Val Tyr Pro Thr Phe Lys Ala Ala Ile Ala Ser Asn Tyr Arg Trp Leu
 340 345 350
 Glu Ala Met Gly Lys Ala Ser
 355

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1884 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: both
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

AGCTTCACTT CGGTTTTATA TTGTGACCAT GGTTCCCAGG CATCTGCTCT AGGGAGTTTT 60
 TCCGCTGCCT TTAGAGAGTA TTTTCTCCAA GTCGGCTAAC TCCCCATTT TTAGGCAAAA 120

- 50 -

TCATATACAG	ACTATCCCAA	TATTGCCAGA	GCTTTGATGA	CTCACTGTAG	AAGGCAGACT	180
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CTAACAGCGG	AAAGAATTAA	ATTTACCCAG	AAACGGGGGT	TTCGTCGGGT	ACTAAACCAA	360
CGGGTGGATG	CCTACTTTGC	CGAGCATGGC	CTGACCCAAA	GGGATAATCC	CTCCATGTAT	420
CTGAAAACCC	TGATTATTGT	GCTCTGGTTG	TTTTCCGCTT	GGGCCTTTGT	GCTTTTTGCT	480
CCAGTTATTT	TTCCGGTGCG	CCTACTGGGT	TGTATGGTTT	TGGCGATCGC	CTTGGCGGCC	540
TTTTCTTCA	ATGTCGGCCA	CGATGCCAAC	CACAATGCCT	ATTCTCTCAA	TCCCCACATC	600
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CGCCACAAC	ATTTGCACCA	CACCTACACC	AATATTCTTG	GCCATGACGT	GGAAATCCAT	720
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CAATTTTATA	TTTGGGGTTT	ATATCTTTTC	ATTCCTTTT	ATTGGTTTCT	CTACGATGTC	840
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GAATTAGCTA	GTTTGCTAGG	GATTAAGCTA	TTATGGCTCG	GCTACGTTTT	CGGCTTACCT	960
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CTCACCCCG	ATGGTGAATC	CGGTGCCATT	GATGACGAGT	GGGCTATTTG	CCAAATTCGT	1140
ACCACGGCCA	ATTTTGCCAC	CAATAATCCC	TTTTGGAACT	GGTTTTGTGG	CGGTTTAAAT	1200
CACCAAGTTA	CCCACCATCT	TTTCCCCAAT	ATTTGTCATA	TTCATATCC	CCAATTGGAA	1260
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CCACTTTGAG	GGGGTTCATT	GGCCGCAGTT	TCAAGCTGAC	CTAGGAGGCA	AAGATTGGGT	1560
GATTTTGCTC	AAATCCGCTG	GGATATTGAA	AGGCTTCACC	ACCTTTGGTT	TCTACCCTGC	1620
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CATGTGGTCT	AACCCAGCCC	TGGCCAAGGC	TTGGACCAAG	GCCATGCAAA	TTCTCCACGA	1740
GGCTAGGCCA	GAAAAATTAT	ATTGGCTCCT	GATTTCTTCC	GGCTATCGCA	CCTACCGATT	1800

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TTTGAGCATT TTTGCCAAGG AATTCTATCC CCACTATCTC CATCCCACTC CCCC GCCTGT 1860
ACAAAATTTT ATCCATCAGC TAGC 1884

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1685 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: both
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

AATATCTGCC TACCCTCCCA AAGAGAGTAG TCATTTTTCA TCAATGGCTG CTCAAATCAA 60
GAAATACATT ACCTCAGATG AACTCAAGAA CCACGATAAA CCCGGAGATC TATGGATCTC 120
GATTCAAGGG AAAGCCTATG ATGTTTCGGA TTGGGTGAAA GACCATCCAG GTGGCAGCTT 180
TCCCTTGAAG AGTCTTGCTG GTCAAGAGGT AACTGATGCA TTTGTTGCAT TCCATCCTGC 240
CTCTACATGG AAGAATCTTG ATAAGTTTTT CACTGGGTAT TATCTTAAAG ATTACTCTGT 300
TTCTGAGGTT TCTAAAGATT ATAGGAAGCT TGTGTTTGAG TTTTCTAAAA TGGGTTTGTA 360
TGACAAAAAA GGTCAATATTA TGTTTGCAAC TTTGTGCTTT ATAGCAATGC TGTTTGCTAT 420
GAGTGTTTAT GGGGTTTTGT TTTGTGAGGG TGTTTTGGTA CATTTGTTTT CTGGGTGTTT 480
GATGGGGTTT CTTGGATTG AGAGTGGTTG GATTGGACAT GATGCTGGGC ATTATATGGT 540
AGTGTCTGAT TCAAGGCTTA ATAAGTTTAT GGGTATTTTT GCTGCAAATT GCTTTTCAGG 600
AATAAGTATT GGTGTTGGA AATGGAACCA TAATGCACAT CACATTGCCT GTAATAGCCT 660
TGAATATGAC CCTGATTTAC AATATATACC ATTCCTTGTT GTGTCTTCCA AGTTTTTTGG 720
TTCCTCACC TCTCATTTCT ATGAGAAAAG GTTGACTTTT GACTCTTTAT CAAGATTCTT 780
TGTAAGTTAT CAACATTGGA CATTTTACCC TATTATGTGT GCTGCTAGGC TCAATATGTA 840
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GTTTGAGAAA CAAACGGATG GGACACTTGA CATTTCTTGT CCTCCTTGGA TGGATTGGTT 1140
TCATGGTGA TTGCAATTCC AAATTGAGCA TCATTTGTTT CCAAGATGC CTAGATGCAA 1200

- 52 -

CCTTAGGAAA ATCTCGCCCT ACGTGATCGA GTTATGCAAG AAACATAATT TGCCTTACAA 1260
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 GCAGGCTAGG GATATAACCA AGCCGCTCCC GAAGAATTTG GTATGGGAAG CTCTTCACAC 1380
 TCATGGTTAA AATTACCCTT AGTTCATGTA ATAATTTGAG ATTATGTATC TCCTATGTTT 1440
 GTGTCTTGTC TTGGTTCTAC TTGTTGGAGT CATTGCAACT TGTCTTTTAT GGTATTATTAG 1500
 ATGTTTTTTA ATATATTTTA GAGGTTTTGC TTTCATCTCC ATTATTGATG AATAAGGAGT 1560
 TGCATATTGT CAATTGTTGT GCTCAATATC TGATATTTTG GAATGTACTT TGTACCACTG 1620
 TGTTTTCAGT TGAAGCTCAT GTGTACTTCT ATAGACTTTG TTAAATGGT TATGTCATGT 1680
 TATTT 1685

(2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 448 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Met	Ala	Ala	Gln	Ile	Lys	Lys	Tyr	Ile	Thr	Ser	Asp	Glu	Leu	Lys	Asn
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			20					25					30		
Asp	Val	Ser	Asp	Trp	Val	Lys	Asp	His	Pro	Gly	Gly	Ser	Phe	Pro	Leu
			35				40					45			
Lys	Ser	Leu	Ala	Gly	Gln	Glu	Val	Thr	Asp	Ala	Phe	Val	Ala	Phe	His
			50			55					60				
Pro	Ala	Ser	Thr	Trp	Lys	Asn	Leu	Asp	Lys	Phe	Phe	Thr	Gly	Tyr	Tyr
					70					75					80
Leu	Lys	Asp	Tyr	Ser	Val	Ser	Glu	Val	Ser	Lys	Asp	Tyr	Arg	Lys	Leu
					85				90					95	
Val	Phe	Glu	Phe	Ser	Lys	Met	Gly	Leu	Tyr	Asp	Lys	Lys	Gly	His	Ile
					100			105					110		
Met	Phe	Ala	Thr	Leu	Cys	Phe	Ile	Ala	Met	Leu	Phe	Ala	Met	Ser	Val
					115		120					125			
Tyr	Gly	Val	Leu	Phe	Cys	Glu	Gly	Val	Leu	Val	His	Leu	Phe	Ser	Gly
						135					140				

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Cys Leu Met Gly Phe Leu Trp Ile Gln Ser Gly Trp Ile Gly His Asp
 145 150 155 160
 Ala Gly His Tyr Met Val Val Ser Asp Ser Arg Leu Asn Lys Phe Met
 165 170 175
 Gly Ile Phe Ala Ala Asn Cys Leu Ser Gly Ile Ser Ile Gly Trp Trp
 180 185 190
 Lys Trp Asn His Asn Ala His His Ile Ala Cys Asn Ser Leu Glu Tyr
 195 200 205
 Asp Pro Asp Leu Gln Tyr Ile Pro Phe Leu Val Val Ser Ser Lys Phe
 210 215 220
 Phe Gly Ser Leu Thr Ser His Phe Tyr Glu Lys Arg Leu Thr Phe Asp
 225 230 235 240
 Ser Leu Ser Arg Phe Phe Val Ser Tyr Gln His Trp Thr Phe Tyr Pro
 245 250 255
 Ile Met Cys Ala Ala Arg Leu Asn Met Tyr Val Gln Ser Leu Ile Met
 260 265 270
 Leu Leu Thr Lys Arg Asn Val Ser Tyr Arg Ala Gln Glu Leu Leu Gly
 275 280 285
 Cys Leu Val Phe Ser Ile Trp Tyr Pro Leu Leu Val Ser Cys Leu Pro
 290 295 300
 Asn Trp Gly Glu Arg Ile Met Phe Val Ile Ala Ser Leu Ser Val Thr
 305 310 315 320
 Gly Met Gln Gln Val Gln Phe Ser Leu Asn His Phe Ser Ser Ser Val
 325 330 335
 Tyr Val Gly Lys Pro Lys Gly Asn Asn Trp Phe Glu Lys Gln Thr Asp
 340 345 350
 Gly Thr Leu Asp Ile Ser Cys Pro Pro Trp Met Asp Trp Phe His Gly
 355 360 365
 Gly Ser Gln Phe Gln Ile Glu His His Leu Phe Pro Lys Met Pro Arg
 370 375 380
 Cys Asn Leu Arg Lys Ile Ser Pro Tyr Val Ile Glu Leu Cys Lys Lys
 385 390 395 400
 His Asn Leu Pro Tyr Asn Tyr Ala Ser Phe Ser Lys Ala Asn Glu Met
 405 410 415
 Thr Leu Arg Thr Leu Arg Asn Thr Ala Leu Gln Ala Arg Asp Ile Thr
 420 425 430
 Lys Pro Leu Pro Lys Asn Leu Val Trp Glu Ala Leu His Thr His Gly
 435 440 445

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(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 8 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Trp Ile Gly His Asp Ala Gly His
1 5

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 8 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Asn Val Gly His Asp Ala Asn His
1 5

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 8 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Val Leu Gly His Asp Cys Gly His
1 5

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 8 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

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Val Ile Ala His Glu Cys Gly His
1 5

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 8 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Val Ile Gly His Asp Cys Ala His
1 5

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 8 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Val Val Gly His Asp Cys Gly His
1 5

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 5 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

His Asn Ala His His
1 5

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

His Asn Tyr Leu His His
1 5

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

His Arg Thr His His
1 5

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

His Arg Arg His His
1 5

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(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 5 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

His Asp Arg His His
1 5

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 5 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

His Asp Gln His His
1 5

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 5 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

His Asp His His His
1 5

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 5 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

His Asn His His His
1 5

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Phe Gln Ile Glu His His
1 5

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

His Gln Val Thr His His
1 5

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 5 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

His Val Ile His His
1 5

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 5 amino acids
- (B) TYPE: amino acid

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(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

His Val Ala His His
1 5

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

His Ile Pro His His
1 5

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

His Val Pro His His
1 5

1 WHAT IS CLAIMED:

1. An isolated nucleic acid encoding a borage $\Delta 6$ -desaturase.

5

2. The isolated nucleic acid of Claim 1 comprising the nucleotide sequence of SEQ ID NO: 4.

10 3. An isolated nucleic acid that codes for the amino acid sequence of SEQ ID NO: 5.

4. A vector comprising the nucleic acid of any one Claims 1-3.

15 5. An expression vector comprising the isolated nucleic acid of any one of Claims 1-3 operably linked to a promoter and optionally a termination signal capable of effecting expression of the gene product of said isolated nucleic acid.

20

6. The expression vector of Claim 5 wherein said promoter is a Δ -6 desaturase promoter, an Anabaena carboxylase promoter, a helianthinin promoter, a glycinin promoter, a napin promoter, the 35S promoter from CaMV, or
25 a helianthinin tissue-specific promoter.

7. The expression vector of Claim 5 wherein said promoter is constitutive or tissue-specific.

30 8. The expression vector of Claim 5 wherein said termination signal is a Synechocystis termination

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1 signal, a nopaline synthase termination signal, or a seed
termination signal.

5 9. A cell comprising the vector of any one of
Claims 4-8.

10 10. The cell of Claim 9 wherein said cell is an
animal cell, a bacterial cell, a plant cell or a fungal
cell.

11. A transgenic organism comprising the
isolated nucleic acid of any one of Claims 1-3.

15 12. A transgenic organism comprising the vector
of any one of Claims 4-8.

20 13. The transgenic organism of Claim 11 or 12
wherein said organism is a bacterium, a fungus, a plant or
an animal.

24. A plant or progeny of said plant which has
been regenerated from the plant cell of Claim 10.

25 15. The plant of Claim 14 wherein said plant is
a sunflower, soybean, maize, tobacco, peanut, carrot or
oil seed rape plant.

30 16. A method of producing a plant with
increased gamma linolenic acid (GLA) content which
comprises:

1 (a) transforming a plant cell with the isolated
nucleic acid of any one of Claims 1-3; and

(b) regenerating a plant with increased GLA
content from said plant cell.

5

17. A method of producing a plant with
increased gamma linolenic acid (GLA) content which
comprises:

(a) transforming a plant cell with the vector of
10 any one of Claims 4-8; and

(b) regenerating a plant with increased GLA
content from said plant cell.

18. The method of Claim 16 or 17 wherein said
15 plant is a sunflower, soybean, maize, tobacco, peanut,
carrot or oil seed rape plant.

19. A method of inducing production of gamma
linolenic acid (GLA) in an organism deficient or lacking
20 in GLA which comprises transforming said organism with the
isolated nucleic acid of any one of Claims 1-3.

20. A method of inducing production of gamma
linolenic acid (GLA) in an organism deficient or lacking
25 in GLA which comprises transforming said organism with the
vector of any one of Claims 4-8.

21. A method of inducing production of gamma
linolenic acid (GLA) in an organism deficient or lacking
30 in GLA and linoleic acid (LA) which comprises transforming
said organism with an isolated nucleic acid encoding

35

1 borage $\Delta 6$ -desaturase and an isolated nucleic acid encoding
 $\Delta 12$ -desaturase.

22. The method of Claim 21 wherein said
5 isolated nucleic acid encoding $\Delta 6$ -desaturase comprises
nucleotides 44 to 1390 of SEQ. ID NO: 4.

23. A method of inducing production of
octadecatetraeonic acid in an organism deficient or
10 lacking in gamma linolenic acid which comprises
transforming said organism with the isolated nucleic acid
of any one of Claims 1-3.

24. A method of inducing production of
15 octadecatetraeonic acid in an organism deficient or
lacking in gamma linolenic acid which comprises
transforming said organism with the vector of any one of
Claims 4-8.

25. The method of Claim 23 or 24 wherein said
20 organism is a bacterium, a fungus, a plant or an animal.

26. A method of producing a plant with improved
chilling resistance which comprises:

25 (a) transforming a plant cell with the isolated
nucleic acid of any one of Claims 1-3; and

(b) regenerating said plant with improved
chilling resistance from said transformed plant cell.

27. A method of producing a plant with improved
30 chilling resistance which comprises:

35

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1 (a) transforming a plant cell with the vector of
any one of Claims 4-8; and

 (b) regenerating said plant with improved
chilling resistance from said transformed plant cell.

5

28. The method of Claim 26 or 27 wherein said
plant is a sunflower, soybean, maize, tobacco, peanut,
carrot or oil seed rape plant.

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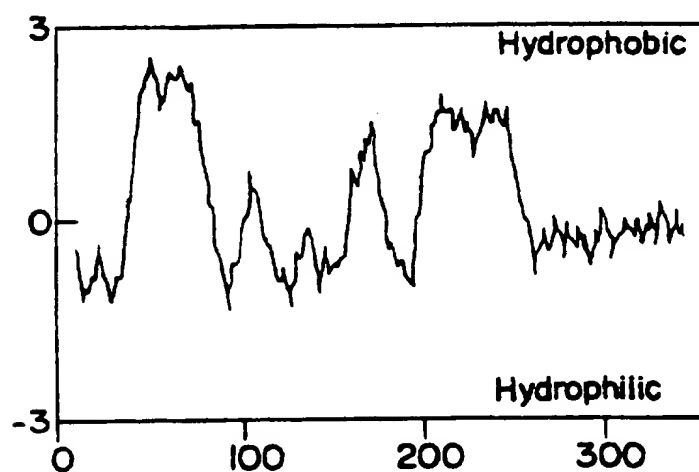


FIG. IA

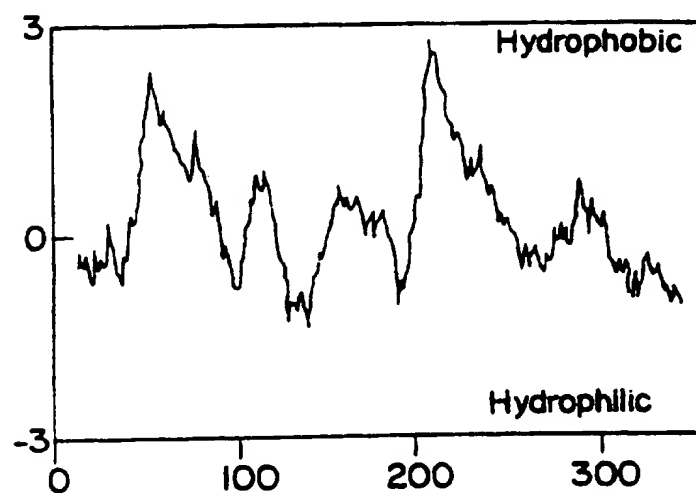


FIG. IB

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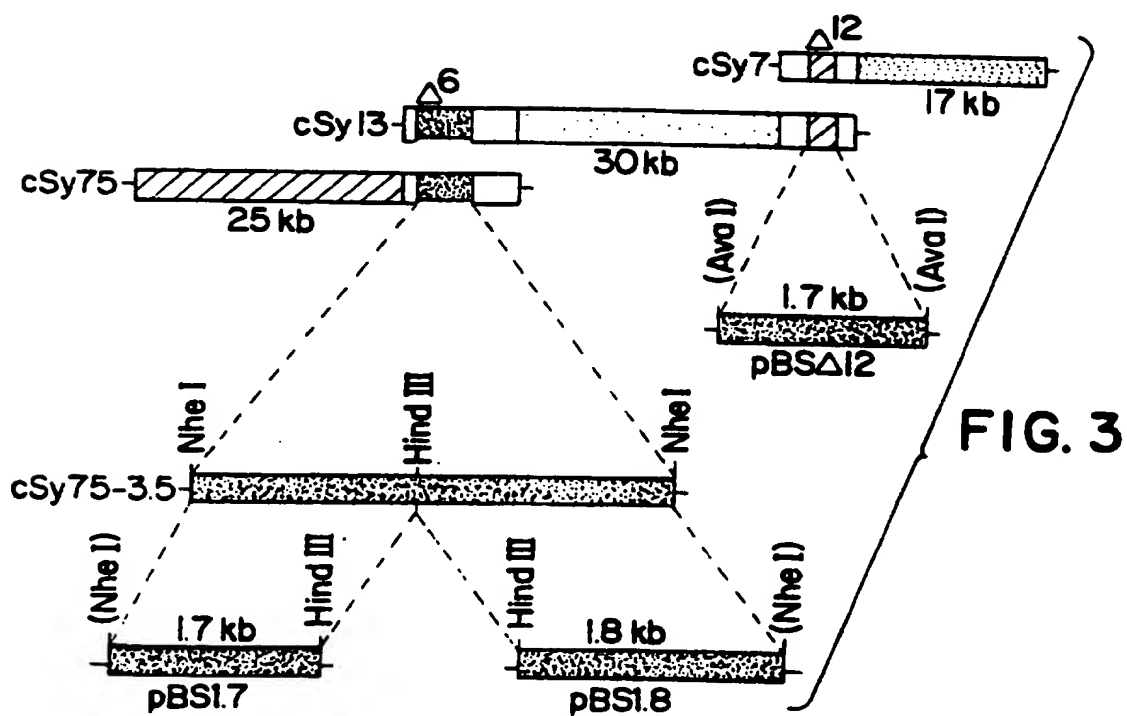
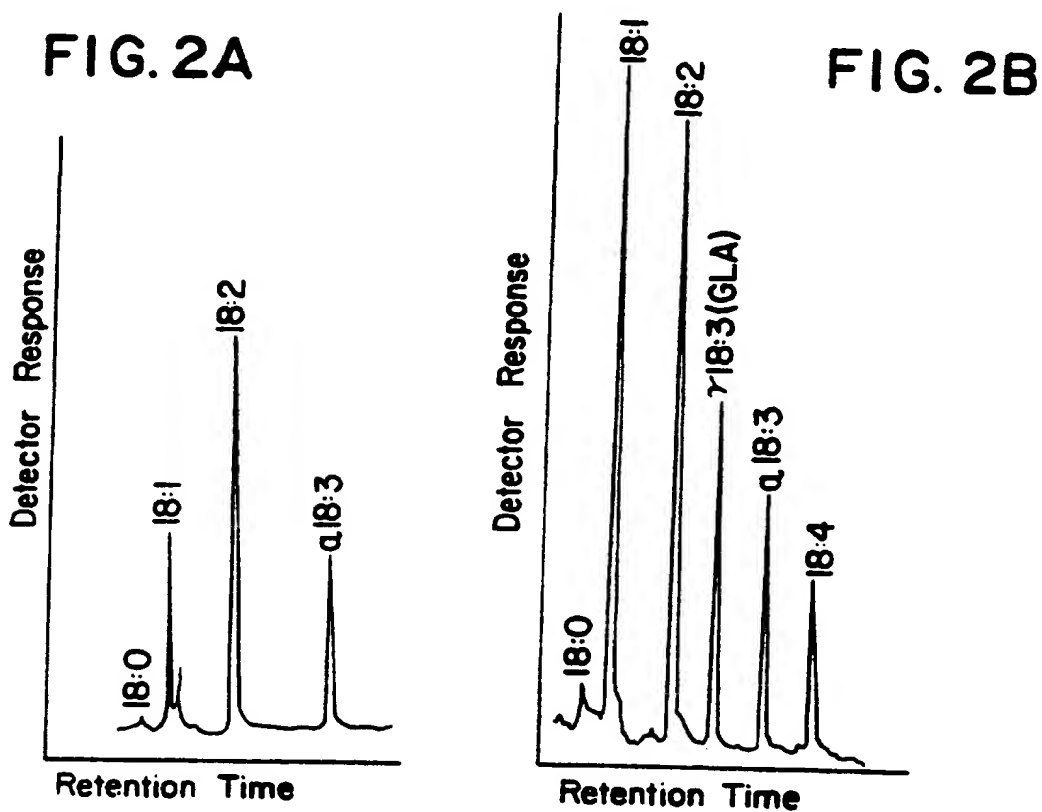


FIG. 3

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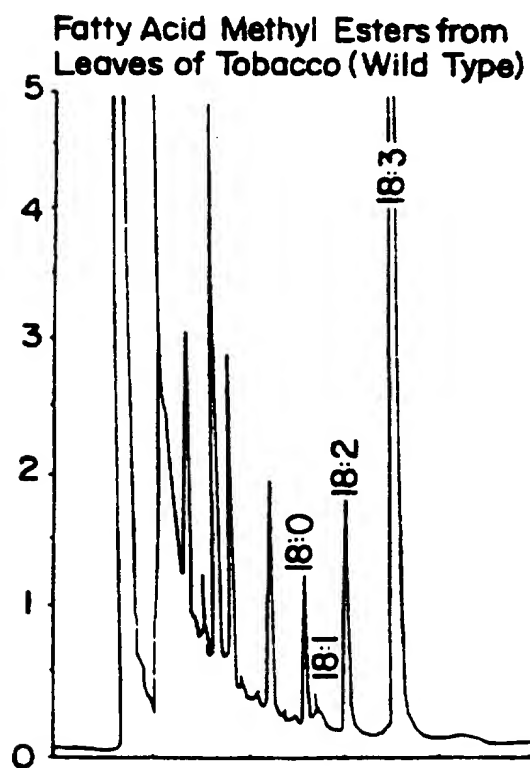


FIG. 4A

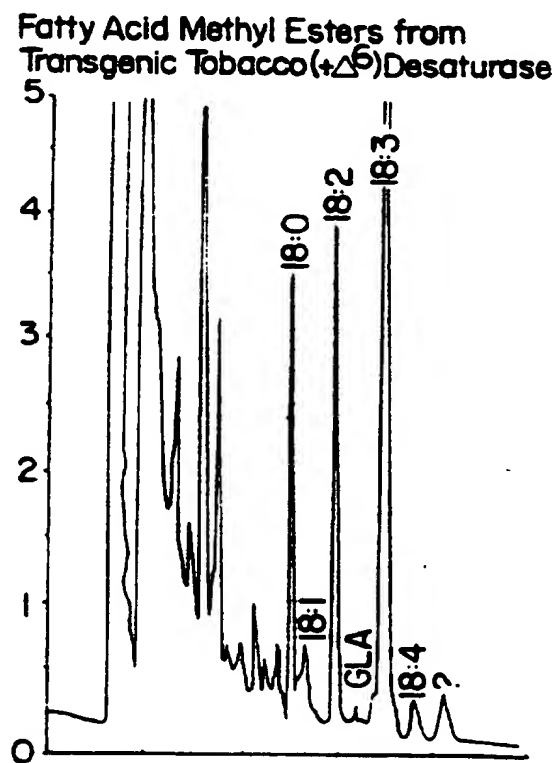


FIG. 4B

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FIG.5A

1 aatatctgcc taccctccca aagagagtag tcatTTTTtca tcaatggctg ctcaaatcaa gaaatacatt acctcagatg 80
 81 aactcaagaa ccacgataaa cccggagatc tatggatctc gattcaaggg aaagcctatg atgtttcgga ttgggtgaaa 160
 161 gaccatccag gtggcagctt tcccttgaag agtcttgctg gtcaagaggt aactgatgca ttgttgcat tccatcctgc 240
 241 ctctacatgg aagaatcttg ataatTTTT cactgggtat tatctaaag attactctgt ttctgaggtt tctaaagatt 320
 321 ataggaaagt tgtgtttgag ttttctaaaa tgggttttga tgacaaaaa ggtcatatta tgtttgcaac tttgtgcttt 400
 401 atagcaatgc tgtttgctat gagtgtttat ggggttttgt ttgtgaggg tgttttgga catttgttt ctgggtggtt 480
 481 gatggggttt ctltggattc agagtgggtg gattggacat gatgctgggc attatatggt agtgtctgat tcaaggctta 560
 561 ataatgttat ggttatTTTT gctgcaaat gcttttcagg aataagtatt ggttggtgga aatggaacca taatgcacat 640
 641 cacattgcct gtaatagcct tgaatatgac cctgattttac aatatatacc attccttgtt gtgtcttcca agttttttgg 720
 721 ttcactcacc tctcatttct atgagaaaag gttagctttt gactctttat caagatttct tttaagtatt caacattgga 800
 801 cattttacc tattatgtgt gctgctaggc tcaatatgta tgtacaatct ctcataatgt tgttgacca gagaaatgtg 880
 881 tccatctgag cttaggaact cttaggatgc ctagtgttct cgatttgga ccggttgctt gtttcttgtt tgcctaattg 960
 961 ggtgaaaga attatgtttg ttattgcaag ttatcagt actggaatgc aacaagtcca gttctccttg accacttct 1040
 1041 ctccaagtgt ttatgtttga aagcctaaa ggaataatg gtttgaaaa caaacggatg ggacacttga catttcttgt 1120
 1121 cctccttga tggattggtt tcatggtgga ttgcaattcc aaattgagca tcatttgttt cccaagatgc cttagtgcaa 1200
 1201 ccttaggaaa atctgcctt acgtgatcga gttatgcaag aaacataatt tgccttaaaa ttatgcatct ttctccaagg 1280
 1281 ccaatgaaat gacactcaga acattgagga acacagcatt gcaggctagg gatataacca agcgcctccc gaagaatttg 1360
 1361 gtatgggaag ctcttcacac tcatggttaa aattaccctt agttcatgta ataatttgag attatgtatc tctatgttt 1440
 1441 gtgtcttgct ttggttctac ttgttgagat cattgcaact tgccttttat ggtttattag atgtttttta atatatatta 1520
 1521 gaggttttgc tttcatctcc attattgatg aataaggagt tgcataattgt caattgttgt gctcaatatac tgatattttg 1600
 1601 gaatgtactt tgtaccactg tgttttcagt tgaagctcat gtgtacttct atagactttg tttaaatggt tatgtcatgt 1680
 1681 tattt 1685

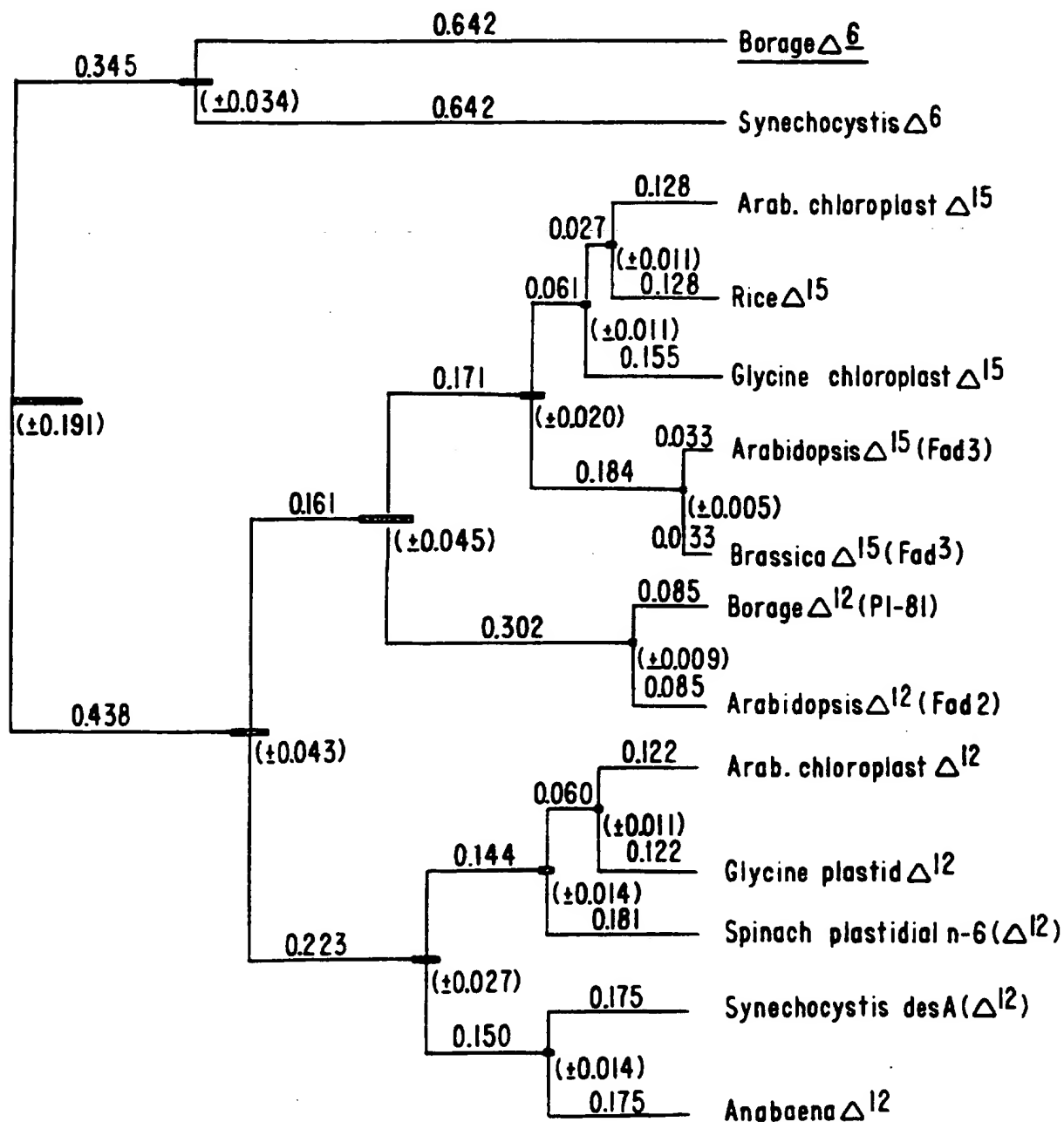
FIG.5B

1 MAAQIKKYIT SDELKNHDKP GDLWISIQGK AYDVSDWVKD HPGGSFPLKS LAGQEVTDAF VAFHPASTWK NLDKFFTGYY 80
 81 LKDYSVSEVS KDYRKLVEEF SKMGLYDKKG HIMFATLCFI AMLFAMSVYG VLFCEGVLVH LFSGLMGFL WIOSGWIGHD 160
 161 AGHYMVVSDS RLNKFMGIFA ANCLSGISIG WKKWNHNAHH IACNSLEYDP DLQYIPFLVV SSKFFGSLTS HFYEKRLTFD 240
 241 SLRFFVSYQ HWTFYPIMCA ARLNMVQSL IMLLTKRNV YRAQELLGCL VFSIWYPLL V SCLPNWGERI MFVIASLSVT 320
 321 GMOQVQFSLN HFSSSVYVGK PKGNWFEKQ TDGTLDISCP PWDWFHGGGL QFOIHHHLP KMPRCNLRKI SPYVIELCKK 400
 401 HNLPPNYASF SKANEMTLRT LRNTALQARD ITKPLPKNLV WEALTHG 448

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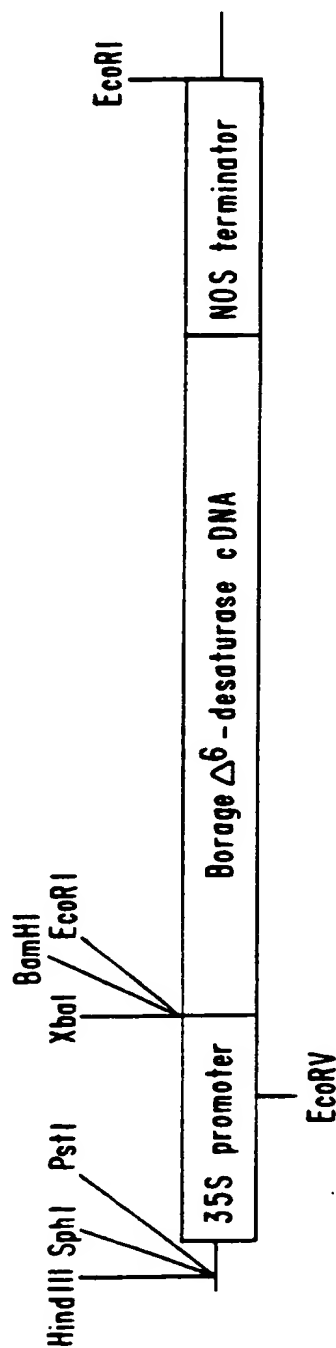
FIG. 6



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FIG. 7



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FIG. 8A

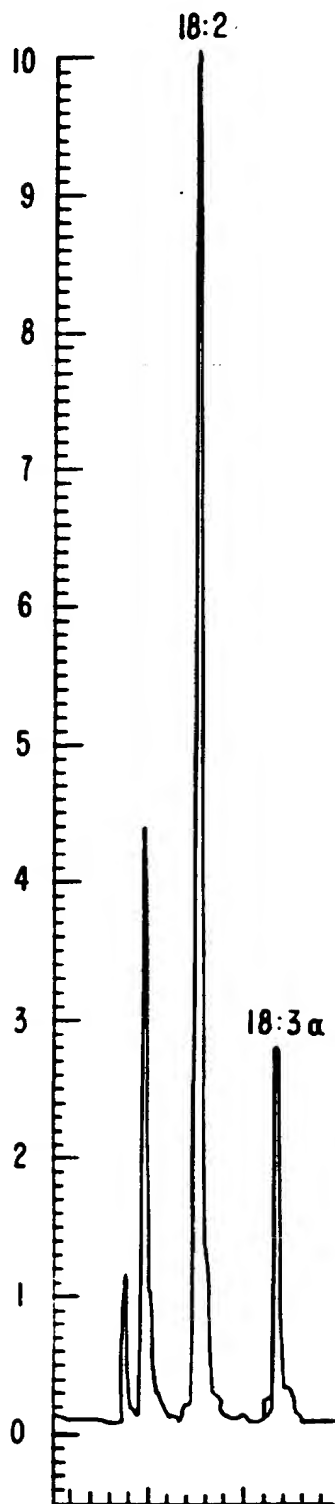
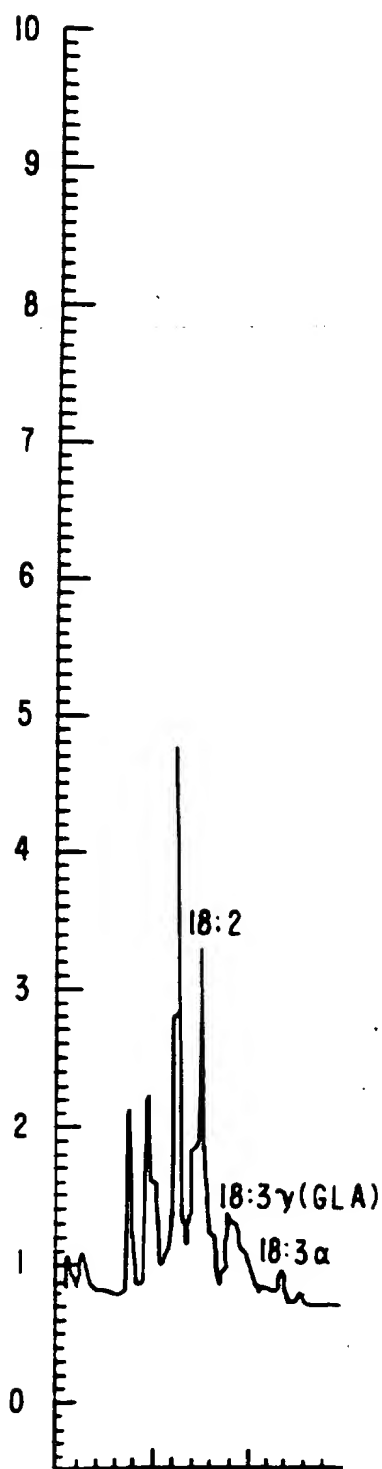


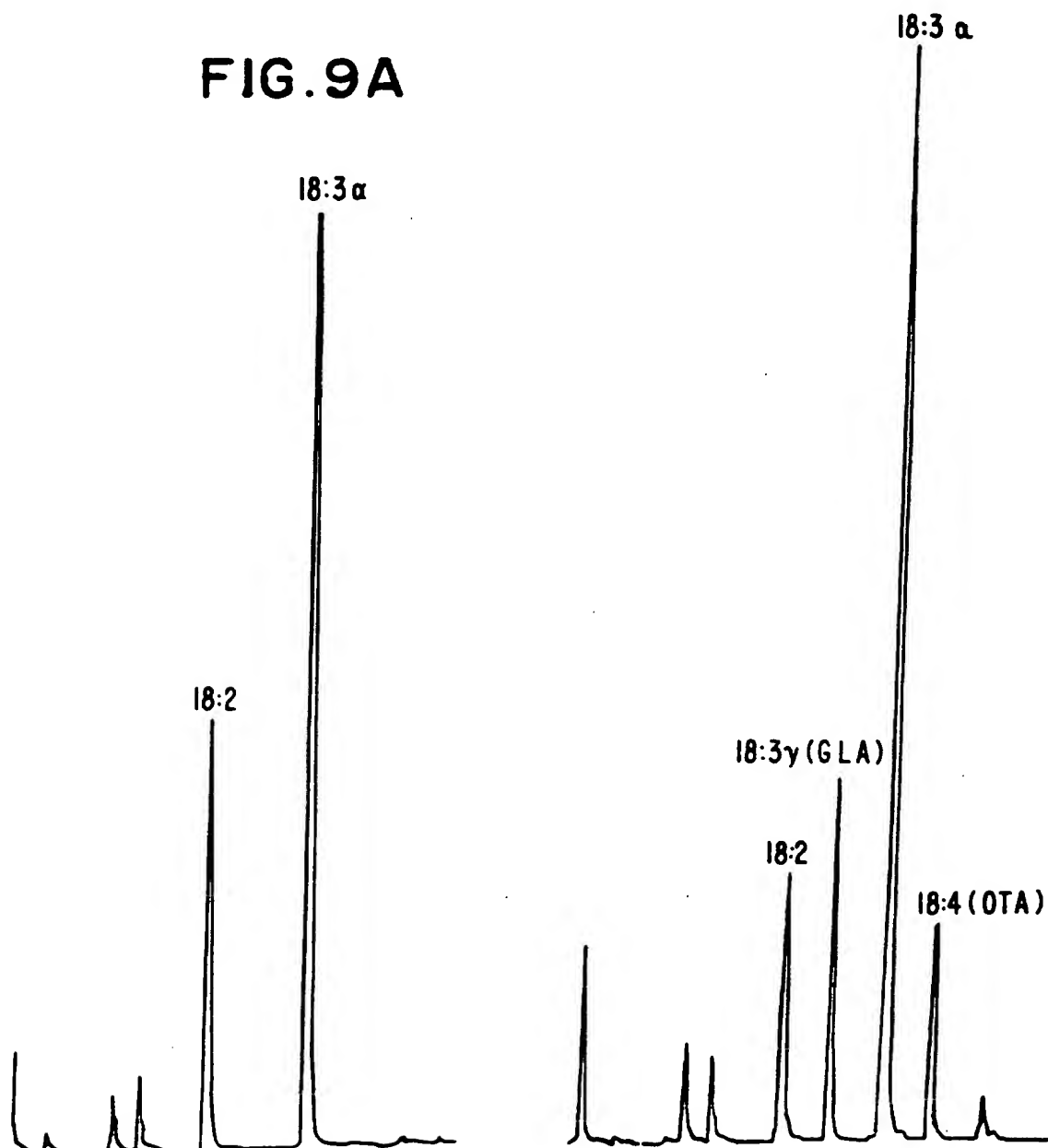
FIG. 8B



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FIG. 9B

FIG. 9A



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FIG. 10A

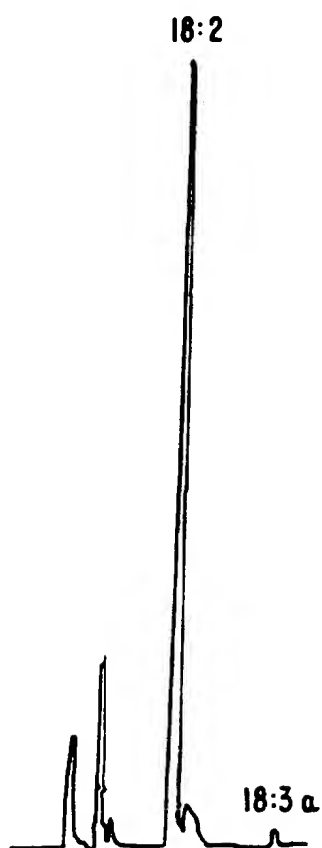
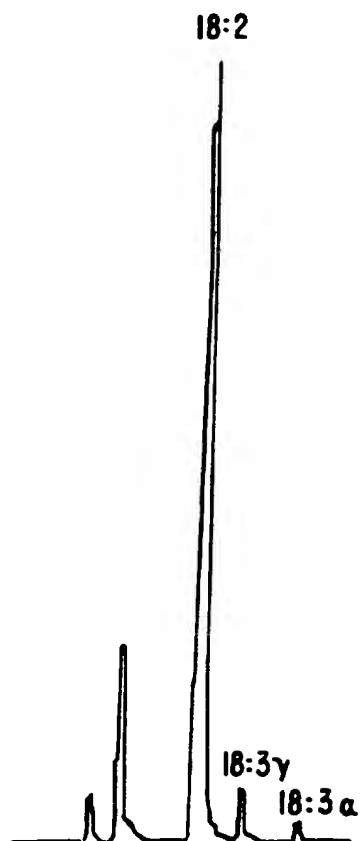


FIG. 10B



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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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			(43) International Publication Date: 11 July 1996 (11.07.96)
(21) International Application Number: PCT/IB95/01167		(81) Designated States: AU, BR, CA, CN, JP, RO, RU, UA, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).	
(22) International Filing Date: 28 December 1995 (28.12.95)			
(30) Priority Data: 08/366,779 30 December 1994 (30.12.94) US		Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>	
(71) Applicant: RHONE-POULENC AGROCHIMIE [FR/FR]; 14- 20, rue Pierre-Baizet, F-69263 Lyon (FR).		(88) Date of publication of the international search report: 12 September 1996 (12.09.96)	
(72) Inventors: THOMAS, Terry, L.; 3004 Normand, College Station, TX 77845 (US). REDDY, Avutu, S.; 3902 E. 29th Street #G11, Bryan, TX 77802 (US). NUCCIO, Michael; P.O. Box 553, College Station, TX 77841 (US). NUNBERG, Andrew, N.; 2804 B. Sprucewood Street, Bryan, TX 77801 (US). FREYSSINET, Georges, L.; 21, rue de Nervieux, F-69450 Saint-Cyr-au-Mont-d'Or (FR).			
(74) Agent: MITSCHERLICH & PARTNER; Sonnenstrasse 33, D- 80331 München (DE).			
(54) Title: PRODUCTION OF GAMMA LINOLENIC ACID BY A $\Delta 6$ -DESATURASE			
(57) Abstract			
<p>Linoleic acid is converted into γ-linolenic acid by the enzyme $\Delta 6$-desaturase. The present invention is directed to isolated nucleic acids comprising the $\Delta 6$-desaturase gene. More particularly, the isolated nucleic acid comprises the promoter, coding region and termination regions of the $\Delta 6$-desaturase gene. The present invention provides recombinant constructions comprising the $\Delta 6$-desaturase coding region in functional combination with heterologous regulatory sequences. The nucleic acids and recombinant constructions of the instant invention are useful in the production of GLA in transgenic organisms.</p>			

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ES	Spain	ML	Mali	UG	Uganda
FI	Finland	MN	Mongolia	US	United States of America
FR	France	MR	Mauritania	UZ	Uzbekistan
GA	Gabon			VN	Viet Nam

INTERNATIONAL SEARCH REPORT

National Application No.

PCT/IB 95/01167

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/53 C12N15/82 A01H5/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N A01H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<p>KADER, J.-C. AND P. MAZLIAK (ED.). PLANT LIPID METABOLISM; 11TH INTERNATIONAL MEETING ON PLANT LIPIDS, PARIS, FRANCE, JUNE 26-JULY 1, 1994. XX+588P. KLUWER ACADEMIC PUBLISHERS: DORDRECHT, NETHERLANDS; NORWELL, MASSACHUSETTS, USA. 0 (0). 1995. 509-511. ISBN: 0-7923-3250-4, XP000569979</p> <p>GALLE A-M, ET AL.: "Solubilization of DELTA-12- and DELTA-6-desaturases from seeds of borage microsomes." see the whole document</p> <p style="text-align: center;">---</p> <p style="text-align: center;">-/--</p>	1-28

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

*** Special categories of cited documents:**

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
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- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *&* document member of the same patent family

Date of the actual completion of the international search

4 July 1996

Date of mailing of the international search report

23.07.96

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentaan 2
NL - 2280 HV Rijswijk
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Fax: (+ 31-70) 340-3016

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Maddox, A

INTERNATIONAL SEARCH REPORT

International Application No

PCT/IB 95/01167

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	KADER, J.-C. AND P. MAZLIAK (ED.). PLANT LIPID METABOLISM; 11TH INTERNATIONAL MEETING ON PLANT LIPIDS, PARIS, FRANCE, JUNE 26-JULY 1, 1994. XX+588P. KLUWER ACADEMIC PUBLISHERS: DORDRECHT, NETHERLANDS; NORWELL, MASSACHUSETTS, USA. 0 (0). 1995. 21-23. ISBN:0-7923-3250-4, XP000569981 SCHMIDT H, ET AL.: "PCR-based cloning of membrane-bound desaturases" see the whole document ---	1-3
Y	WO,A,93 06712 (RHONE POULENC AGROCHIMIE) 15 April 1993 see the whole document ---	4-28
A	BIOCHEM J 252 (3). 1988. 641-648. , XP000568812 GRIFFITHS G: "DELTA-6 AND DELTA-12 DESATURASE ACTIVITIES AND PHOSPHATIDIC ACID FORMATION IN MICROSOMAL PREPARATIONS FROM THE DEVELOPING COTYLEDONS OF COMMON BORAGE BORAGO-OFFICINALIS." see the whole document ---	1-3
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A	PLANT MOLECULAR BIOLOGY, vol. 26, 1994, pages 631-642, XP002000999 SCHMIDT, H., ET AL.: "Purification and PCR-based cDNA cloning of a plastidial n-6 desaturase" see the whole document ---	1-3
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INTERNATIONAL SEARCH REPORT

International Application No.

PCT/IB 95/01167

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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A	NATURE, vol. 347, 13 September 1990, pages 200-203, XP002001001 WADA, H., ET AL.: "Enhancement of chilling tolerance of a cyanobacterium by genetic manipulation of fatty acid desaturation" see the whole document ---	26-28
A	PLANT PHYSIOLOGY, vol. 105, no. 2, June 1994, pages 601-605, XP002001002 KODAMA, H., ET AL.: "Genetic enhancement of cold tolerance by expression of a gene for chloroplast omega-3 fatty acid desaturase in transgenic tobacco" see the whole document -----	26-28

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No.

PCT/IB 95/01167

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		AU-B- 2881292	03-05-93
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